



**Newcastle**  
University

# **Lead levels in teeth as a measure of life-time lead exposure in children**

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# ABSTRACT

## Background and aims

Lead exposure has irreversible health effects in children who are susceptible even at very low levels of exposure. The usual test for lead exposure is blood lead level (BLL), but this indicates only recent exposure. This study aimed to ascertain the suitability of milk teeth as biomarker of the history of lead exposure and to develop a methodology for this novel biomarker.

## Methods

My study comprised three stages: Firstly, I explored potential determinants of dentine lead levels (DLLs) in children living in Newcastle upon Tyne (the Tooth Fairy Study). Secondly, I developed a methodology for assessing the history of early life lead exposure using dentine, and thirdly I applied my methodology to newly extracted teeth from children in Teesside.

The Tooth Fairy study cohort consisted of 69 children aged 5-8 years. DLLs were measured in primary dentine using Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS). To identify determinants of early life exposure, a questionnaire was used. I assessed associations between lifestyle characteristics and DLLs.

As teeth develop chronologically they offer the opportunity to study histories of exposure in detail. I collected two deciduous molars each from 15 children aged 6-8 years living in Northeast England. By combining high spatial resolution LA-ICP-MS with dental histology, I acquired information on the age specific concentrations of lead in dentine from *in utero* to several years after birth.

## Results

Dentine lead levels in the Tooth Fairy cohort ranged from 0.06 to 0.77µg/g, median 0.21µg/g. Unlike other studies, I did not find significant associations between socio-economic status or other possible determinants and lead exposure.

In developing the biomarker I found that the 100 micron ablation pit represented 42 days of dentine growth, enabling me to assign an age interval to each ablation pit. I found lead ratios in primary dentine to be consistent between teeth from the same child, and at the same age within each tooth. This indicated that the history of exposure can be determined using a single, multi-point ablation transect on high quality longitudinal sections of individual teeth.

## **Conclusions**

- I found no association between socio-economic status and dentine lead levels in a cohort of children from Newcastle upon Tyne.
- I developed a novel technique to date ablation points in dentine in milk teeth, and, using this technique have demonstrated that primary dentine is a potential biomarker for characterising the early life history of lead exposure in children.

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# STATEMENT OF CONTRIBUTION

This is to certify that the work contained in this thesis comprises original work conducted by the student under supervision of Professor Tanja Pless-Mulloli, Dr Susan Hodgson, Dr Wendy Dirks and Dr Tom Shepherd. My own contributions and those of others were as follows:

- For the epidemiological study I used survey data collected prior to my arrival. I carried out all statistical analysis of these data.
- Dr Paul Averley and his team at Queensway Dental Clinic assisted in the recruitment process and with sample collection of teeth and blood samples in Teesside. Blood samples were analysed at the Health and Safety Laboratory, Buxton. I used the blood lead data for my analysis
- For the histological study I carried out all measurements.
- For the measurement of lead I prepared the teeth with the assistance of Pam Walton in the School of Dental Sciences, Newcastle University. The lead analysis was carried out by Dr Tom Shepherd and his team at the University of Leeds. I used all data to interpret with the histological dating.
- The thesis has not been submitted for the award of any other degree at any other institution.

## JOURNAL PUBLICATIONS AND CONFERENCE PAPERS

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2. Manmee C, Hodgson S, Dirks W, Shepherd T, Pless-Mulloli T. (2011) Lead levels in teeth as a measure of life-time lead exposure in children. *American Journal of Physical Anthropology*, 2011: 144, Supplement 52:205. Presented at The 2011 AAPA Symposium in Honor of Don Reid. *Evolutionary Anthropology*. 2011; 20:161-163. (Poster presentation, 80<sup>th</sup> Annual meeting of the American Association of Physical Anthropologists (AAPA), Minneapolis, Minnesota, USA, April 2011)
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8. Dirks W, Shepherd TJ, Manmee C, Hodgson S, Averley P, Pless-Mulloli T. (in prep) Daily incremental growth lines in dentine.

9. Manmee C, Hodgson S, Dirks W, Shepherd TJ, Pless-Mullooli T. (in prep)  
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# TABLE OF CONTENTS

<b>ABSTRACT</b>	<b>ii</b>
<b>ACKNOWLEDGEMENTS</b>	<b>iv</b>
<b>STATEMENT OF CONTRIBUTION</b>	<b>vi</b>
<b>JOURNAL PUBLICATIONS AND CONFERENCE PAPERS</b>	<b>vii</b>
<b>TABLE OF CONTENTS</b>	<b>ix</b>
<b>LIST OF TABLES</b>	<b>xiv</b>
<b>LIST OF FIGURES</b>	<b>xv</b>
<b>ABBREVIATIONS</b>	<b>xviii</b>
<b>CHAPTER ONE: INTRODUCTION</b>	<b>1</b>
1.1 Introduction	2
1.2 Aim and objectives	3
1.3 Structure of the thesis	3
<b>CHAPTER TWO: SCIENTIFIC BACKGROUND</b>	<b>5</b>
2.1 Environmental and health impacts of lead exposure	6
2.1.1 Introduction	6
2.1.2 History of Lead Exposure	7
2.1.3 Sources of Lead Exposure	8
2.1.4 Exposure pathways	14
2.1.5 Fate of lead in the human body	16
2.1.6 Lead and Health Effects	19
2.1.6.1 Acute effects	19
2.1.6.2 Chronic effects	20
2.1.7 Global distribution for human exposure to lead	24
2.1.8 Factors influencing human lead exposure	25
2.1.8.1 Demographic factors	26
2.1.8.2 Socio-economic and occupational factors	27
2.1.8.3 Hand to mouth behaviour and soil	28
2.1.8.4 Residential areas and house conditions	29
2.1.8.5 Dietary intake	29

2.1.8.6 Health	30
2.1.8.7 Other factors	31
<b>2.2 Biological monitoring of trace elements</b>	<b>32</b>
2.2.1 Introduction	32
2.2.2 Biomarkers for monitoring lead exposure	33
2.2.2.1 Blood and plasma	33
2.2.2.2 Urine and Feaces	34
2.2.2.3 Saliva	35
2.2.2.4 Sweat	36
2.2.2.5 Hair	36
2.2.2.6 Nail	38
2.2.2.7 Bone	38
2.2.2.8 Teeth	39
<b>2.3 Tooth morphology, development and histology</b>	<b>41</b>
2.3.1 Tooth development (Odontogenesis)	41
2.3.2 Dental histology	44
2.3.2.1 Enamel	44
2.3.2.2 Dentine	48
2.3.3 Age-related characteristics of dental histology	52
2.3.4 Lead incorporation in enamel and dentine	54
2.3.4.1 Enamel mineralisation	54
2.3.4.2 Lead levels in enamel	55
2.3.4.3 Dentine mineralisation	56
2.3.4.4 Lead levels in dentine	58
<b>2.4 Measurement of lead in teeth and blood</b>	<b>60</b>
2.4.1 Lead levels in teeth	60
2.4.2 Tooth-lead levels and health outcome	61
2.4.3 Blood lead levels	61
2.4.4 Correlation between tooth and blood lead levels	62
<b>2.5 Principles of the method used in this study</b>	<b>64</b>
2.5.1 Introduction	64
2.5.2 Inductively Coupled Plasma Mass Spectrometry (ICP-MS)	64
2.5.2.1 Principles and Components of ICP-MS	65
2.5.2.2 Advantages and Limitations of ICP-MS	66

2.5.3 Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS)	67
2.5.3.1 Principles and Components of LA-ICP-MS	67
2.5.3.2 Advantages and Limitations of LA-ICP-MS	68
<b>CHAPTER THREE: MATERIALS AND METHODS</b>	<b>70</b>
<b>3.1 Study design</b>	<b>71</b>
<b>3.2 Phase I: The Tooth Fairy epidemiological study</b>	<b>71</b>
3.2.1 Setting	72
3.2.2 Ethical approval	74
3.2.3 Selection criteria and sample size	74
3.2.4 Methods of data collection	74
3.2.4.1 Recruitment	74
3.2.4.2 Collection of sample teeth	75
3.2.4.3 Questionnaire	75
3.2.5 Data storage	76
3.2.6 Analysis of the teeth	77
3.2.7 Statistical analyses	78
3.2.7.1 Hypotheses	78
3.2.7.2 Descriptive analyses	78
3.2.7.3 Multivariate analyses	78
<b>3.3 Phase II: Development of histological technique</b>	<b>79</b>
3.3.1 Introduction	79
3.3.2 Participant recruitment and selection criteria	79
3.3.3 Ethical approval	79
3.3.3.1 Consent	80
3.3.3.2 Confidentiality and data storage	80
3.3.4 The process of the collection of teeth and blood samples	81
3.3.5 Preparation of teeth for the histological study	82
3.3.5.1 Tooth catalogue	83
3.3.5.2 Tooth sectioning	83
3.3.5.3 Tooth lapping	85

3.3.6 Identification of sections for initial laser ablation	87
3.3.7 LA-ICP-MS analysis of the sample teeth	92
3.3.8 Age measurement at each ablation point	94
3.3.9 Analysis strategy	100
<b>3.4 Phase III: Application of the histological technique to remaining teeth sample</b>	<b>101</b>
 <b>CHAPTER FOUR: RESULTS</b>	 <b>104</b>
<b>4.1 Characteristics of the study participants</b>	<b>105</b>
4.1.1. Tooth Fairy Study	105
A: Participants and their main place of residence	105
B: Characteristics of child's eating and drinking behaviour and health	109
C: Socio-economic status and occupational characteristics	110
D: Dentine lead levels in children from the Tooth Fairy Study	11
4.1.2 Details of participants recruited into the Teesside samples	113
<b>4.2 Question 1: Are mean DLLs associated with known determinants of lead exposure?</b>	<b>114</b>
4.2.1 Univariate analyses	114
4.2.2 Multivariate analyses	126
<b>4.3 Question 2: Can our histological technique recreate the history of lead exposure of over a child's life time?</b>	<b>128</b>
4.3.1 Question 2a: Should we sample enamel and/or dentine?	128
4.3.2 Question 2b: How many transects per tooth are required?	133
4.3.3 Question 2c: How many teeth per child are required?	137
4.3.4 Question 2d: Does tooth type matter?	148
<b>4.4. Question 3: What is the relationship between tooth and blood lead levels?</b>	<b>152</b>
 <b>CHAPTER FIVE: DISCUSSION AND CONCLUSIONS</b>	 <b>156</b>
<b>5.1 Discussion</b>	<b>157</b>
5.1.1 Epidemiological study	157
5.1.2 Histological study	167



5.1.3 Overall discussion	175
5.1.4 Strengths and limitations of the epidemiological study	177
5.1.5 Strengths and limitations of the histological study	179
<b>5.2 Conclusions</b>	<b>181</b>
5.2.1 Epidemiological study	181
5.2.2 Histological study	182
<b>5.3 Recommendations</b>	<b>184</b>
<b>5.4 Direction of future work</b>	<b>185</b>
 <b>REFERENCES</b>	 <b>187</b>
<b>APPENDICES</b>	<b>215</b>
<b>APPENDIX I. THE NEWCASTLE TOOTH FAIRY STUDY'S</b>	<b>216</b>
<b>QUESTIONNAIRE</b>	
<b>APPENDIX II. CONSENT FORM</b>	<b>229</b>
<b>APPENDIX III. PUBLICATIONS</b>	<b>230</b>
<b>APPENDIX IV. POSTER PRESENTATIONS</b>	<b>242</b>

## LIST OF TABLES

<b>Table 2.1</b> Estimated daily lead intake and absorption in adults	17
<b>Table 2.2</b> Summary of the half-life of lead in each biomarker	40
<b>Table 2.3</b> Comparison of lead levels in whole primary teeth from different countries	60
<b>Table 3.1</b> Characteristics of tooth samples in the histological study	84
<b>Table 3.2</b> Laser operating parameters and related ICP-MS conditions	93
<b>Table 3.3</b> Conversion of age intervals into age in days for enamel and dentine	100
<b>Table 4.1</b> Characteristics of children and their main place of residence	107
<b>Table 4.2</b> Details of fifteen Teesside children recruited in the histological study	113
<b>Table 4.3</b> Associations between child household characteristic and DLLs	120
<b>Table 4.4</b> Multiple regression analysis examining the determinants of DLLs	126

## LIST OF FIGURES

<b>Figure 2.1</b> Sources and pathways for environmental lead	10
<b>Figure 2.2</b> Lead emissions by source sector in USA	14
<b>Figure 2.3</b> The source-pathway exposure of environmental contamination and health	16
<b>Figure 2.4</b> Primary dentitions (occlusal views)	42
<b>Figure 2.5</b> Component and structure of the tooth	43
<b>Figure 2.6</b> Incremental enamel features	46
<b>Figure 2.7</b> Neonatal line in longitudinal section of a deciduous tooth	47
<b>Figure 2.8</b> The cross striations in enamel in the present study	47
<b>Figure 2.9</b> The regions of dentine	49
<b>Figure 2.10</b> Long period lines or Andresen lines in dentine	51
<b>Figure 2.11</b> Short period lines or von Ebner's lines in dentine in the present study	51
<b>Figure 2.12</b> The pattern of dentine mineralization	57
<b>Figure 2.13</b> Element distributions in deciduous incisors	59
<b>Figure 2.14</b> Acceptable BLLs proposed by agencies at different times	62
<b>Figure 2.15</b> Diagram of basic components of ICP-MS	66
<b>Figure 2.16</b> LA-ICP-MS coupled with plasma source	68
<b>Figure 3.1</b> Map showing locations of school divided by wards and IMD scores	73
<b>Figure 3.2</b> Sampling ablation points on the tooth section in the Tooth Fairy study	77
<b>Figure 3.3</b> The desired plane of sectioning for upper deciduous molar	84
<b>Figure 3.4</b> A flow diagram of the sample collection, preparation and analyses	86
<b>Figure 3.5</b> The preparation of the sample teeth	87
<b>Figure 3.6</b> Pattern of incremental lines in enamel and dentine	89
<b>Figure 3.7</b> Ablation transects for the two teeth sections from child HT 1	90
<b>Figure 3.8</b> Ablation transects for the two teeth sections from child HT11	91
<b>Figure 3.9</b> The laser ablation was coupled with the ICP-MS	94
<b>Figure 3.10</b> Age measurement at ablation point in postnatal enamel	97
<b>Figure 3.11</b> Age measurement at ablation point in primary dentine	97
<b>Figure 3.12</b> Age measurement using accentuated lines in postnatal enamel	98
<b>Figure 3.13</b> A chart of deciduous tooth development (Courtesy of Wendy Dirks)	99
<b>Figure 3.14</b> An application of one transect to the remaining teeth	103

## LIST OF FIGURES (continued)

<b>Figure 4.1</b> Mean enamel and dentine lead levels in children recruited in the Tooth Fairy Study	111
<b>Figure 4.2</b> The histogram of mean DLLs in children in the Tooth Fairy study	112
<b>Figure 4.3</b> DLLs by a.household incomes and b.IMD scores of place of residence	118
<b>Figure 4.4</b> Map showing DLLs in children living in Newcastle divided by IMD scores by LSOA	119
<b>Figure 4.5</b> Distribution of DLLs across sampling points in each tooth from the Tooth Fairy study	125
<b>Figure 4.6</b> Distribution of lead ratios across two teeth for child HT1	130
<b>Figure 4.7</b> Comparison of dentine lead ratios by age interval in the two teeth for child HT1	130
<b>Figure 4.8</b> Distribution of lead ratios across two teeth for child HT11	132
<b>Figure 4.9</b> Comparison of dentine lead ratios by age interval in the two teeth for child HT11	132
<b>Figure 4.10</b> A comparison of lead ratios in primary dentine in two teeth for child HT1	136
<b>Figure 4.11</b> A comparison of lead ratios in primary dentine in two teeth for child HT11	137
<b>Figure 4.12</b> Lead ratio distribution for the two teeth from child HT2	138
<b>Figure 4.13</b> Lead ratio in primary dentine for two teeth from child HT2	138
<b>Figure 4.14</b> Lead ratio distribution for the two teeth from child HT3	139
<b>Figure 4.15</b> Lead ratio in primary dentine for two teeth from child HT3	139
<b>Figure 4.16</b> Lead ratio distribution for the two teeth from child HT5	140
<b>Figure 4.17</b> Lead ratio in primary dentine for two teeth from child HT5	140
<b>Figure 4.18</b> Lead ratio distribution for the two teeth from child HT8	141
<b>Figure 4.19</b> Lead ratio in primary dentine for child HT8 tooth 85	141
<b>Figure 4.20</b> Lead ratio distribution for the two teeth from child HT9	142
<b>Figure 4.21</b> Lead ratio in primary dentine for two teeth from child HT9	142
<b>Figure 4.22</b> Lead ratio distribution for the two teeth from child HT12	143
<b>Figure 4.23</b> Lead ratio in primary dentine for two teeth from child HT12	143
<b>Figure 4.24</b> Lead ratio distribution for the two teeth from child HT13	144
<b>Figure 4.25</b> Lead ratio in primary dentine for the two teeth from child HT13	144
<b>Figure 4.26</b> Lead ratio distribution for the two teeth from child HT14	145

## LIST OF FIGURES (continued)

<b>Figure 4.27</b> Lead ratio in primary dentine for the two teeth from child HT14	145
<b>Figure 4.28</b> Comparison of primary dentine lead ratios of all samples from two teeth each child	147
<b>Figure 4.29</b> Comparison of primary dentine lead ratios among children with tooth type 54	149
<b>Figure 4.30</b> Comparison of primary dentine lead ratios among children with tooth type 55	149
<b>Figure 4.31</b> Comparison of primary dentine lead ratios among children with tooth type 64	150
<b>Figure 4.32</b> Comparison of primary dentine lead ratios among children with tooth type 65	150
<b>Figure 4.33</b> Comparison of primary dentine lead ratios among children with tooth type 84	151
<b>Figure 4.34</b> Comparison of primary dentine lead ratios among children with tooth type 85	151
<b>Figure 4.35</b> Scatter plot of blood and enamel lead levels of children from the Teeside sample	153
<b>Figure 4.36</b> Scatter plot of blood and primary dentine lead levels of children from the Teeside sample	153
<b>Figure 4.37</b> The spatial distribution of lead ratios in a primary mandibular left first molar for HT6-74 from the child with the highest BLL	155

## ABBREVIATIONS

AAS	Atomic Absorption Spectrophotometry
AEA	Atomic Energy Authority
AES	Atomic Emission Spectrometry
AFS	Atomic Fluorescence Spectrometry
ALA	American Lung Association
ASV	Anodic Stripping Voltammetry
ATSDR	Agency for Toxic Substances and Disease Registry
BLLs	Blood Lead Levels
BMI	Body Mass Index
CDC	Centers for Disease Control and Prevention
CEJ	Cemento-Enamel Junction
CLG	Communities and Local Government
DDI	Distilled Deionised Water
DDLs	Dentine lead levels
EDJ	Enamel-Dentine Junction
EPA	Environmental Protection Agency
FAAS	Flame Atomic Absorption Spectroscopy
FDI	Federation Dentaire Internationale
GIS	Geographic Information System
GFR	Glomerular Filtration Rate
HEPA	A High-Efficiency Particulate Air
HPA	Health Protection Agency
HSL	Health and Safety Laboratory
HUD	The U.S. Department of Housing and Urban Development
IARC	International Agency for Research on Cancer
ICOH	International Commission on Occupational Health
ICP-AES	Inductively Coupled Plasma Emission
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ICP-OES	Inductively Coupled Plasma-optical Emission Spectrometry
IHS	Institute of Health and Society
IMD	Index of Multiple Deprivation
IQ	Intelligence Quotient
ISIDAS	Interactive Spectral Imaging Data Analysis

## **ABBREVIATIONS (continued)**

LA-ICP-MS	Laser Ablation Inductively Coupled Plasma Mass Spectrometry
LOD	Limit of Determination
LSOA	Lower Layer Super Output Area
MDRD	Modification of Diet in Renal Disease
MS	Mass Spectrometry
NAA	Neutron Activation Analysis
NHANES	National Health and Nutrition Examination Survey
ONS	Office of National Statistics
ppb	Parts per billion
ppm	Parts Per Million
RBCs	Red Blood Cells
REC	Research Ethics Committee
SD	Standard Deviation
SE	Standard Error
SGVs	Soil Guildeline Values
SOA	Super Output Area
SPSS	Statistical Package for the Social Sciences
XRMA	X-Ray Micro-Analyses
WHO	World Health Organisation
µg/dL	Microgram per decilitre
µg/g	Microgram per gram
µg/L	Microgram per litre

# **CHAPTER ONE**

## **INTRODUCTION**



# CHAPTER ONE

## INTRODUCTION

### 1.1 Introduction

Lead is a trace element and common environmental pollutant particularly in industrialised and contaminated areas where elevated lead levels may be found in soil, dust and drinking water. The toxicity of lead to human health has been known for many years. Epidemiological studies have reported that lead can be harmful to health at very low levels of exposure. Compared to adults, children often experience higher exposures and are more susceptible to the health effects associated with lead exposure. Children with elevated lead exposure have been shown to be at risk for deficits in IQ, balance, hearing, and growth and these effects can persist into the teenage and adult years. [1-5]

Among the most important sources of lead exposure for children are ingestion of water from leaded water pipes, paint chips in old buildings in the USA, dermal exposure to lead from toys, certain cosmetic products, and exposure to soil and household dust. Children living in areas with lead contaminated soils can exhibit high lead body burden. [1, 2, 5-7]

The usual method for assessing lead exposure is to measure lead levels in blood, however this can only tell us about recent exposure, over the preceding weeks. [5] In this study I aimed to develop a method to assess long term lead exposure in children using tooth lead levels to reveal lead exposure over the early years of a child's life. I first used standard techniques to explore the determinants of lead exposures as measured in enamel and dentine in the Newcastle Tooth Fairy cohort. Building on these findings I used histological analysis of transects of teeth from Teesside to develop a method to assess the history of lead exposure.

## 1.2 Aim and objectives

The overall aim of my study was to assess levels of lead in different parts of human deciduous teeth to establish a biological marker for studying long term lead exposure.

### **The specific objectives were:**

1. To investigate factors, including socio-demographic characteristics, home and neighbourhood environment, family and occupational exposure, and the child's behaviour that might influence cumulative lead exposure as measured by tooth lead levels in children from Newcastle upon Tyne (the Tooth Fairy Study)
2. To study the histology of dentine to refine and better understand the tooth lead levels to develop a more sensitive biomarker to describe the history of lead exposure using teeth
3. To determine lead levels in different parts of the teeth including enamel and dentine using LA-ICP-MS
4. To assess lead levels in teeth from the same child, and between children to explore intra- and inter-individual variability
5. To explore the correlations between tooth and blood lead levels

## 1.3 Structure of the thesis

This thesis is composed of five chapters. **This first chapter** is an overall introduction to my thesis. **The second chapter** outlines the scientific background and consists of five sections. It starts with an introduction to environmental and health impacts of lead exposure (Section 2.1), followed by with a more detailed description of biological monitoring of trace elements (Section 2.2). The third section (Section 2.3) is a general description of tooth morphology, development and histology, followed by the measurement of lead in teeth and blood (Section 2.4). The last section (Section 2.5) provides a general description of principles of the methods used in this study.

**Chapter three** describes the main materials and methods used. The first section (Section 3.1) gives information about the study design. The next section (Section 3.2) provides details of the first phase of the study, an epidemiological study (the Tooth Fairy Study) to identify determinants of lead exposure, this is followed by the second phase, the development of histological techniques using dentine to identify the chronology of tooth

lead levels (Section 3.3). The last section (Section 3.4) describes the application of the histological technique to the remaining teeth sample.

**Chapter four** presents the results and consists of four sections. It starts with the characteristics of the study participants (Section 4.1). It continues with the results for the main research questions in the epidemiological study: Question one: Are mean dentine lead levels (DLLs) associated with known determinants of lead exposure? (Section 4.2). The results of the subsequent sample development of histological techniques in initial four teeth addressing the research question, including intra- and inter- individual lead levels are presented in Section 4.3, the question was: Can the histological technique recreate the history of lead exposure of over child's life time? The last section describes the relationship between tooth and blood lead levels. (Section 4.4).

**The fifth chapter** discusses the findings of my study in five sections. In the first section (Section 5.1) the determinants of lead exposure in the Tooth Fairy Study are discussed. The following section, 5.2, describes the histological study, and is followed by overall discussion (Section 5.3). I then discuss the strengths and limitations of the epidemiological study (Section 5.4), and histological study (Section 5.5). This is followed by a summary and conclusions. Recommendationa and future directions of research based on the scientific results of the present study are also described.

# **CHAPTER TWO**

## **SCIENTIFIC BACKGROUND**

# **CHAPTER TWO**

## **SCIENTIFIC BACKGROUND**

### **2.1 Environmental and health impacts of lead exposure**

#### **2.1.1 Introduction**

Lead is a common environmental pollutant and its health impacts are globally recognised as a public health problem.[3] The Agency for Toxic Substances and Disease Registry (ATSDR) identified lead as one of the most hazardous substances in the environment. [8] In addition, the Secretary of the Department of Health and Human Services in the USA determined in 1991 that ‘Lead is the number one environmental pollutant which is harmful to US children’. [9] Exposure to lead can have detrimental effects on a person’s intelligence and social behaviour well into teenage and adult years. [10, 11] More recently it has been reported that lead can be harmful at very low levels of exposure, [4, 12] even below the current guideline values.

The determination of health risks to and effects on individuals of environmental lead exposure requires the use of accurate and credible biomarkers. [5, 13] Blood lead levels are used routinely for monitoring lead exposure in humans in some countries; however, lead levels in blood have a short half-life, a maximum of 40 days [14] This means that blood is not suitable for identifying long-term exposure.

In this chapter I describe the history of lead exposure, its sources of exposure, the fates of lead in the body, health effects, and epidemiological studies of lead exposure. Issues of tooth development, components, and mineralization are also described. The techniques used to identify blood and tooth lead levels are described in the final section.

## 2.1.2 History of Lead Exposure

Lead was recognised as the father of all metals by the ancients. [15] It is believed that lead was found in Egypt as early as 3500-4000 BC, and it was used in most of the major ancient civilised societies, such as those of Hebrews, Romans and Greeks. [16] Woolley (1984) noted that lead poisoning was one of the earliest occupational diseases to be recognised. [17] During the industrial revolution in the mid eighteenth century, the production of lead increased resulting in many cases of lead poisoning in occupationally exposed workers. Once the amount of lead in the environment increases the incidence of neonatal lead exposure also rises, with associated impacts on pregnancy outcome. [16] Studies suggest that lead exposure in pregnant women is associated with abortion, premature birth, maternal hypertension and reduced fetal growth. [18]

In 1921, one of the most important events in the history of lead exposure occurred: the anti-knock effect of tetraethyl lead was discovered at the Kettering Research Laboratories of General Motors. The subsequent inclusion of lead in petrol resulted in toxic organo-lead compounds increasing global lead emissions due to their volatile nature [19].

In the twentieth century, the USA was the world leader in producing and consuming lead at about 1.3 million tons per year according to a report of Lead in the Human Environment undertaken by the National Academy of Science in 1980. [15] As well as its use as an anti-knocking agent in petrol, lead was also used widely as a major component of pigment in many paints, cosmetics, and household artefacts. The banning of pregnant women from working in the manufacture of lead pigment was one of the very first measures implemented by the British Workplace legislation in 18<sup>th</sup> century. The discovery of a mysterious illness among workers in tetraethyl lead manufacturing attracted interest, and the deaths of workers in refineries in New Jersey and Ohio during 1924 led to an investigation into the cause. Lead poisoning was eventually recognised as the cause, which resulted in lead poisoning being recognised as an occupational hazard for those with lead exposure. [20] In the 1970s, the U.S. Environmental Protection Agency (EPA) began to implement proper regulations for lead use. Restrictions for lead use in petrol were started in 1975, and in 1982, unleaded petrol was introduced. As a result of these measures, leaded fuel use dropped 98% between 1970 and 1986 in the USA. [21] Subsequently, lead use in fuel was banned in the USA in 1988. However, it is

still in use in many developing countries until the present day. [22] Due to improvements in industrial hygiene, the incidence of acute lead poisoning has also gradually decreased in most developed countries. In developing countries, on the other hand, the incidence of paediatric lead poisoning remains high. [23]

Lead paint has remained a problem especially in older houses in spite of the phasing out of production in 1978 in the USA and 1992 in Europe. [24] Leaded paint was banned for sale in the UK in 1992. [25] In the early 1970s, the US launched the Lead-Based Paint Poisoning Act in order to develop methods for removing lead paint from houses.

However, studies have indicated that the removal of lead dust from houses and the procedures used to reduce lead in paints were not very effective. The main pathways for exposure to lead from paint are the ingestion of paint chips, the swallowing of lead home dust, and from contaminated soil due to hand-to-mouth behaviour, and therefore these routes of lead exposure are still of concern in young children. [20]

The phasing out of lead use in the UK started in the 1970s, and lead in air has declined dramatically. [26] With regard to body burden in the UK, a study found that BLLs fell substantially between 1984 and 1995. Since 1984-1987, BLLs in adults have decreased by 2.6-3.0  $\mu\text{g/dL}$  [27], and 97% of individuals in 1995 had BLLs lower than 10 $\mu\text{g/dL}$ . [28]

Since 1991, the US Centers for Disease Control and Prevention has determined that the highest acceptable blood lead level in children (BLL) is 10 $\mu\text{g/dL}$ . [29] Nevertheless, evidence suggests that subtle effects can occur at even lower levels. [10, 30, 31] As a result, the USA announced a national policy to decrease BLLs to lower than 10 $\mu\text{g/dL}$  in children in the USA by the year 2010. [29] Over the past three decades there has been increasing awareness of the effects of lead on health. The removal of lead in petrol and paint has resulted in decreasing BLLs in children in the USA and much of Europe. [8, 32]

### **2.1.3 Sources of Lead Exposure**

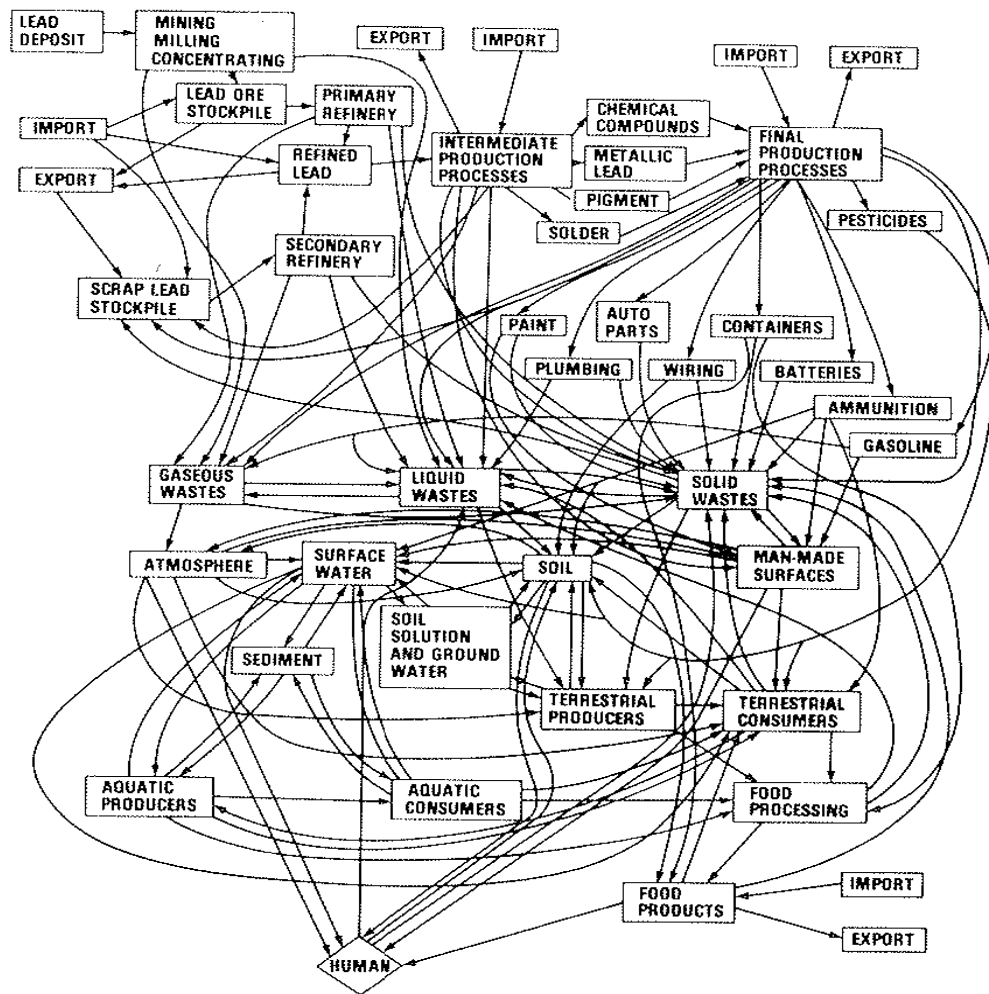
Lead is found in many sources and locations. There are many pathways for exposure to lead, such as exhaust emissions from leaded petrol-driven vehicles (leaded petrol), lead pipes, lead dust and soil, exposure in mining industries, and from pesticides and old buildings with leaded paint.[1, 2, 5-7] However, the connection between environmental

contamination with pollutants such as lead and human exposure is complicated. Figure 2.1 illustrates some of the sources, products, pathways and media of human exposure to lead, highlighting the complex web of lead use, environmental contamination and human exposure.

Historically, the main sources of exposure among children in the USA are airborne lead (generally originating from petrol combustion) and leaded dust and chips derived from the deterioration of lead paint. [1] Leaded petrol remains one of the major sources of lead exposure in developing countries such as Thailand and Indonesia. [33, 34]

Occupational lead exposure is related to industries such as mining, metal smelting, battery manufacture and others that use lead, lead-based paint, plastics, ceramics, radiators, inks, or solder. [23, 35] Some studies have indicated that children toys, cosmetics, and folk medicines may also contain lead.[35] The following sections give greater details of selected sources of environmental lead, including petrol, lead-contaminated dust, lead-based paint, lead in soils, and lead water pipes.





**Figure 2.1** Sources and pathways for environmental lead

From Rutter, M. and Jones, RR. (1983) *Lead versus health : sources and effects of low level lead exposure*. Wiley: Chichester. [2]

## Lead in Petrol

Leaded petrol was first introduced in commercial markets in the USA in 1923. The pioneering manufactures were Dupont and General Motors, who developed tetraethyl lead, which is the highly toxic compound used to boost octane performance in petrol. By the mid 1970s it had become clear that automobile emissions from the use of leaded petrol were a major source of environmental lead contamination. Subsequent studies found adverse health effects due to exposure to lead. The US EPA then regulated lead in petrol, and the phasing out of lead in the vehicles began in 1978. After several years of decreasing leaded-petrol, BLLs in the American survey had dropped 77 percent. The regulation for reducing lead use continued to create more efficient products such as safer fuel additives and efficiency.[20] In the UK, the lead content in petrol decreased from

0.4g/l to 0.15g/l in 1985, subsequently decreased airborne lead levels were found. A study of motor vehicles and air pollution at 29 sites in the UK found that lead concentrations in the air fell at least 50% between 1986 and 1987. [36] The substantial reduction of air lead level was then found due to unleaded petrol until present. [36, 37]

By 1990s, levels of lead in petrol decreased dramatically in developed countries, and subsequently lead petrol usage also decreased in most developing countries. [20] Currently, there are only six countries in the world that still sell leaded petrol; these are: Afghanistan, Algeria, Iraq, North Korea, Myanmar and Yemen. However according to the United Nation's Environment Programme, leaded petrol will be banned around the world within the next two years, and these countries have agreed to stop selling by 2013. [37]

### **Lead-contaminated dust**

Dust is largely composed of particles of small size deriving from soil, paint, plant pollen, textile fibres, and industrial and automotive activities, as well as other materials found in the local environment. In homes these particles settle on exposed surfaces and are trapped in carpets, flooring, curtains and clothing. In exposure studies, it was reported that specks of lead-contaminated dust are virtually impossible to identify visually and even small amounts can be harmful to children. [38, 39] Exposure studies have revealed that for two year old children in the UK urban environment, approximately 50% of the lead found in the body was from dust ingested as a result of hand-to-mouth activity. [40] A survey carried out in 1981 to 1982 measured household dust and garden soils for 100 homes in Newcastle. Lead levels were reported to be approximately 600µg/g and 300µg/g in household dust and garden soils respectively. [41] Research conducted between 1981 and 1992 in the UK concluded that approximately 10% of the population was exposed to lead levels in excess of 2,000µg/g in household dust. [40]

### **Lead-based paint**

Lead paint from old houses has long been determined as a potential source of exposure to lead in the UK. [42] Lead was commonly used in household paint in the UK until the mid-1960s. It was most widely used paint for windows, doors, woodwork, and metal items. Old lead paint poses a danger if the paintwork flakes or peels, is chewed or bitten by children or pets, or when sanded or burnt off in preparation for repainting. When people renovate or repair their older houses or do routine maintenance, they are

potentially at risk of exposure to lead dust. Even a small amount of lead dust or small chips of lead-based paint pose a risk of increased exposure that may impact health. [42] To reduce levels of lead in UK paints, legislation was introduced in the 1970s. Nevertheless, older paints still persist in older houses. The risk following the ingestion of old paint flakes are well known, and the majority of cases of lead poisoning in the UK have been among children who ingested paint flakes or dust contaminated with lead. In the USA, Sutton et al (1995) estimated that there were approximately 1.3 million homes in California in which interior paint lead levels exceeded 5,000 ppm [43] and Kassa et al (2000) described lead poisoning as a significant health problem among some children living in inner-city homes in Toledo, Ohio. [44] Kokori et al (1999) identified lead as an important current environmental health problem threatening infants. [45]

In most developed countries and some developing countries where legislation has been used to control lead-based paints, the hazard posed should decrease over time. However, it is important that people are aware of the risk posed, because it is likely to take several years or decades for old leaded paintwork in residential areas to be entirely removed, and even after removal of paint, contaminated dust and soil remain a resource of potential lead exposure. [42]

## **Lead in soils**

Soil can be an important exposure pathway for humans. Lead is relatively immobile in soils and has been found to accumulate in the top horizons of the soil profile, therefore becoming a long-term source of lead exposure. [46] Moreover, soil is the final resting area of airborne lead from petrol and dust. [8, 47] Paustenbach et al (1997) found that tracked-in or resuspended soil particles were a significant component of household dust. [48]

Some studies assessing the use of soil as a diagnostic tool for environmental conditions that influence health discovered a significant association between levels of lead in soil and BLLs. [29, 49, 50] The CDC reported that BLLs increase 3-7µg/dL for every 1000ppm rise of lead in soil or dust. [29] However, these increases derived from different sources, people and exposure conditions. For example, a study in the USA undertaken by Lanphear et al (1997) reported that if residential soil-lead concentrations increased from background 1ppm to 1000ppm, children's blood lead concentrations increased by 2.4µg/dL. [51] Duggan (1985) estimated an increment of 5µg/dL per 1000µg/g of soil lead. [52] Madhavan (1989) proposed that, for children younger than 12 years of age, an acceptable

value of 600µg/g of soil lead as "safe", this would contribute less than 5µg/dL to total blood lead. [50]

### **Lead water pipes**

A primary source of lead in tap water is household plumbing systems where the pipes and solder connections contain lead. [38] Lead piping is mostly found in old houses, but in new homes in the USA, "lead-free" plumbing containing less than 8% lead is legal. [53] The EPA has identified that the largest sources of lead in water are brass or chrome-plated fixtures and the illegal use of lead solder. [35] Lanphear et al (1998) reported that drinking water containing lead levels greater than 15ppb are correlated with a 14% increase in the incidence of children having BLLs over 10µg/dL. [54]

In the UK, properties constructed before the 1930s had lead water pipes, many of which are still being used. In 1998 the EC Drinking Water Directive (98/83/EC) launched new standards for parameters including lead in drinking water. This standard was first applied in England by the Water Supply (Water Quality) Regulations 2000. This regulation has two phases; from 25 December 2003 water supplied to all premises must not exceed a level of 25 microgrammes of lead per litre of water, while from 25 December 2013, a maximum of 10 microgrammes of lead per litre of water will be introduced. [55]

However, older housing still faces the problem of lead solder used to connect water pipes and lead pipes bringing water from the street supply into the house and to tap/water outlets.

### **Other lead sources**

Hand to mouth behaviour has a major influence on lead exposure, especially in young children who put lead contaminated products such as toys, chalk, jewellery and metallic, soils/dusts, and accessories into their mouths. [35] Pica behavior is a practice to eat non-edible materials/substances such as dirt, clay, and soil. If these are contaminated with lead, children who pick up and eat these objects may receive lead contamination by ingestion.

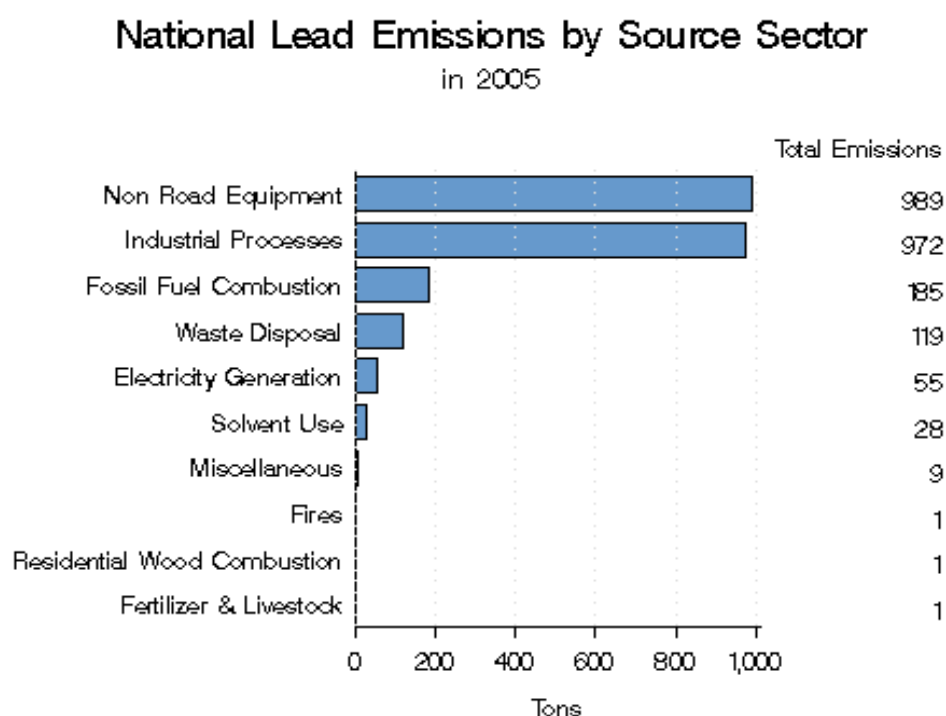
The use of lead oxide glazes in ceramics is also associated with higher blood lead levels. Usually such glazes are not heated to sufficiently high temperatures to remove the lead. Lead then leaches from the pottery and ceramics into food during processes of food

preparation and storage. Lead has also been identified as a contaminant in various home goods, crystal and metallic accessories.

Occupational exposure is another source of lead exposure, especially among those who work in factories using leaded compounds. In the UK, a survey of BLLs in workers between 2008-2009 found that the three industry sectors with the highest number of males working with lead were the lead battery industry (17.5%), the smelting, refining, alloying and casting industry (15.4%), and the metallic lead and lead-containing alloys sector (11.2%). A similar trend was found in female workers. [56]

## 2.1.4 Exposure pathways

Other sources of lead have been identified such as waste disposal, firearms with lead bullets, and wood combustion. A report on national lead emissions in twelve sectors in 2005 undertaken by the U.S. Environmental Protection Agency (USEPA) indicated a range of sources similar to those found in other areas globally [57], as shown in Figure 2.2.

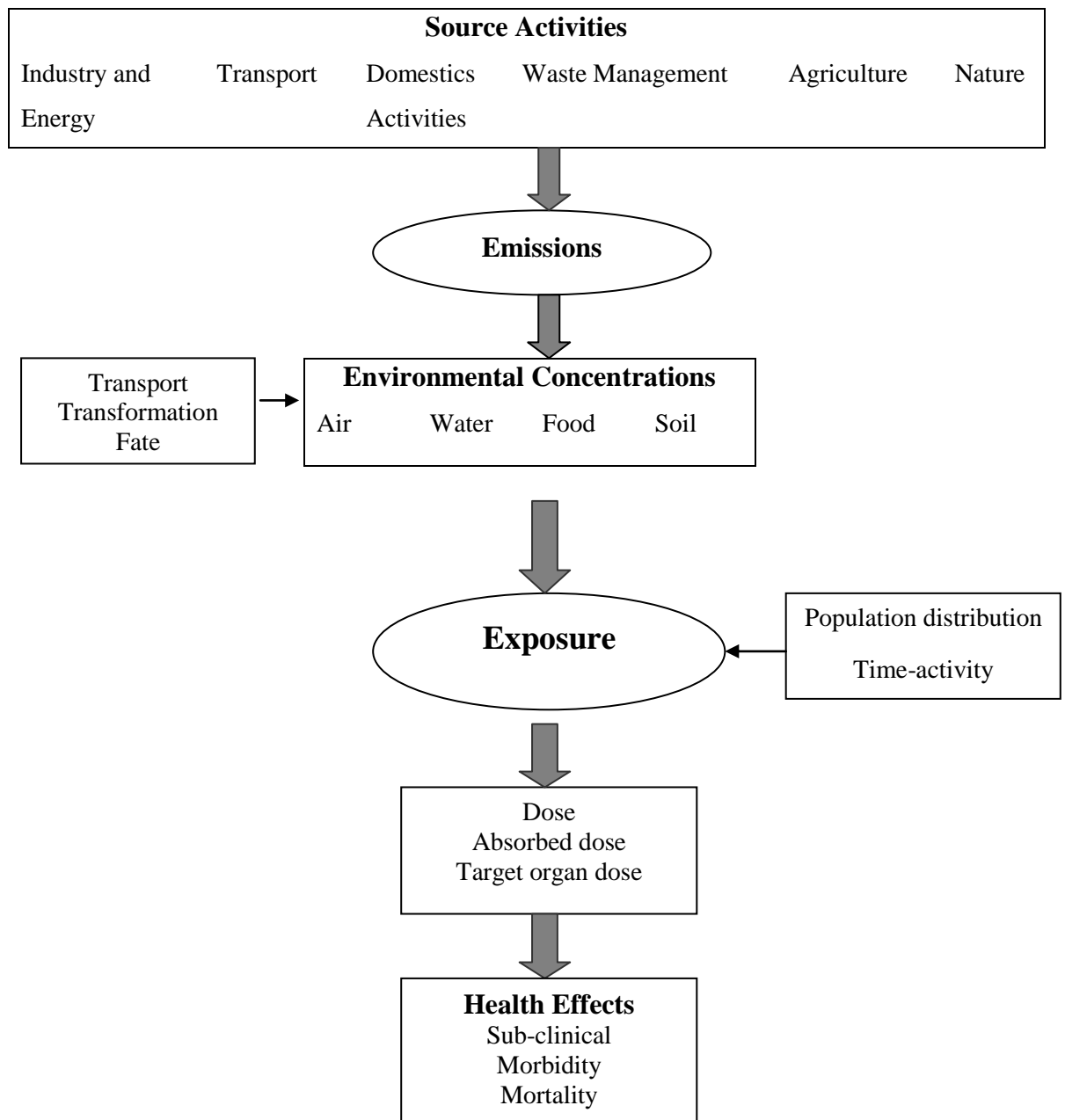


**Figure 2.2** Lead emissions by source sector in USA

From US EPA (2005) ‘National Emissions Inventory Data & Documentation’. [57]

The source-pathway-exposure chain commences with source activities such as industry and energy, transport, and domestic activities. These actions generate emissions into environmental compartments such as air, water, food and soil. These pollutants can result in exposure to humans via direct or indirect pathways. The US Environmental Protection Agency (EPA) has specified the reference levels of lead in environmental media such as air, soil and water based on a level of exposure which is not likely to be harmful to human health, however there is not likely to be a 'safe' level of exposure. The WHO has also identified lead levels in humans via different biomarkers such as blood lead levels.

Lead emissions to air, water, food and soil and can be transported into the human body. The degree of individual exposure depends on dosage, age at exposure, physiological and target organs. Figure 2.3 illustrates the source-pathway exposure model linking of environmental contamination and health.



**Figure 2.3** The source-pathway exposure of environmental contamination and health  
From Briggs, D. (2003) 'Environmental pollution and the global burden of disease'.  
*British Medical Bulletin*. Vol. 68, pp.1–24. [58]

### 2.1.5 Fate of lead in the human body

Lead compounds are generally toxic, and enter the body by several routes such as ingestion, inhalation and skin absorption. [59] Most inorganic lead forms are not well absorbed via the skin, but organic forms such as tetraethyl lead are readily absorbed. [60] The ingestion of contaminated food and water is the main source of lead exposure for the general population and inhalation is the main route of lead exposure in the workplace.[60]

The following sections describe the kinetics of lead in the human body from absorption, distribution, and excretion.

Absorption through the gastrointestinal tract is an important route for lead to enter the human body. Once ingested, lead is readily absorbed through the gastrointestinal tract. Gastric absorption ranges between 5% and 15% in adults though may reach 50% in those with calcium, iron and zinc deficiencies. The uptake of lead in children is usually higher than that in adults. [61] Children aged between 2 months and 6 years can absorb 42 to 50% of an oral dose. The reason for higher lead absorption in children is unclear. A possible explanation is a higher frequency of iron, calcium and phosphate deficiencies in children. In adults, absorption rates decrease to 4-21% when lead is taken with food including normal calcium and phosphate constituents. [59, 61] The estimated relationship between lead intake and absorption is given in Table 2.1.

**Table 2.1** Estimated daily lead intake and absorption in adults [59]

<b>Pathways</b>	<b>Lead intake</b>	<b>% Absorption</b>	<b>Lead absorption</b>
Pb intake with food	200-300 µg/day	10%	20-30 µg/day
Pb contents of drinking water	>20 µg/liter	10%, 2 litre/day	> 4 µg/day
Pb contents of air	1 µg/m <sup>3</sup>	30%, 20 m <sup>3</sup> /day	6 µg/day
Total absorption in adults			30-40 µg/day

Another exposure route is inhalation. 90% of lead oxide is absorbed by the lungs. In developed countries concentrations of lead in air have gradually decreased once the use of leaded petrol was banned; however, the burning of petrol and other lead containing products is still one of the main sources of lead contamination in some areas. Relevant activities included industrial lead smelting, the disposal of discarded batteries and other lead-containing materials, burning of garbage and old painted wood, and the weathering of old lead-containing paints on buildings. The burning of coal and fuel oil also contributes to higher atmospheric levels of lead. [62]

Lead in ambient air is comprised of aerosols of particles which can be absorbed and deposited in the respiratory system. [63] Lead is well absorbed in the lungs at levels between 50 to 70% of respirable lead particles. Moreover, around 30% to 50% of the airborne lead inhaled by adults is retained, approximately 80% of which is gradually absorbed into the blood. [60] The absorption of lead within the respiratory tract depends



on several factors, including airborne concentrations of lead, particle size, solubility and density, and the individual's ventilation rate. [63, 64] Lead-containing particles in the range of 0.5 to 5 $\mu$ m in diameter are probably transported to the alveoli, where the exchange of lead and blood can occur. [65]

Finally, dermal absorption is a term used to describe the transport of chemicals/substances from the outer surface of the skin through the skin. [66] Dermal absorption can follow occupational, environmental, or consumer exposure to chemicals, cosmetics, and pharmaceutical products. [66, 67] Although lead is absorbed through the skin, levels of the dermal absorption of inorganic lead substances are recognised to be lower than those from ingestion and inhalation. Only a few studies have estimated the dermal absorption of lead in humans, and these indicate that lead is absorbed through the skin mainly via extracellular fluids such as sweat and saliva. In an experiment by Stauber et al (1994) investigating the absorption of inorganic lead components, lead nitrate and lead acetate were applied to the skin of participants. After six hours, levels of the lead compounds absorbed through the skin were assessed in the saliva, sweat, blood and urine. Significant increases in lead levels were found in the content of sweat and saliva, but not in blood and urine. [68] Sun et al (2002) conducted a study to determine the percutaneous absorption of four compounds, including lead sulphate, lead oxide, inorganic lead powder, and lead stearate. Lead absorbed through the skin was measured in the upper layers of the stratum corneum of 10 lead-battery workers using the method of skin stripping. Amounts of lead significantly increased on skin strappings of the dorsal hand and the back of the lead workers. The amount of lead on the dorsal hand and in the blood was significantly correlated, and the authors concluded that lead was gradually absorbed into the skin from such occupational exposure. [69]

Once lead enters the human body, it is generally absorbed into the blood and distributed throughout the body. Lead circulates, accumulates and is finally excreted as waste. The amount of lead stored in the body has been identified as 'lead body burden'. Total lead body burden in an individual depends on many factors such as the level of lead exposure, lead levels in storage tissues, exposure duration and excretion rate from the body. With respect to storage tissue, about 95% and 70% of the total lead stored in adults and children is stored in bone, 69% of which can be found in cortical bone, while 23% is found in trabecular bone. [70] Because lead levels in cortical bone have a long half life of approximately 10 years compared to 1 year for trabecular bone, the former can be used to

identify long-term lead exposure. [71, 72] Soft tissues such as the liver, kidney, pancreas and lung absorb around 8% of the dose. Tsuchiya (1979) reported that 90% of absorbed lead is found in bone. [70]

It is known that lead transported into blood and soft tissues can move into the placenta during pregnancy. A study in Australia found that 79% of the lead concentrations in cord blood could be transferred from infant to women's bones during pregnancy. [73] Sources of lead in breast milk include that from maternal bone and the diet. Gulson (1998) indicated that lactation increases the release of lead from bone. [74]

Lead is largely excreted in urine and faeces via the kidneys and the intestine. Glomerular filtration eliminates up to 76% of total lead; 16% is eliminated via bile, pancreatic and intestinal excretion; hairs, nails and perspiration excrete less than 8% of lead. [75] Unabsorbed lead is mainly excreted via the faeces. [70] However, faeces lead levels are not a reliable indicator of lead body burden since they illustrate largely unabsorbed lead dose. Dermal absorbed lead can be excreted by sweating and inhaled lead may be excreted by normal exhalation. Other routes for excretion are via saliva and breast milk. It should be noted that adults can excrete up to 99% of lead over time but children can only excrete 32%, the rest being retained in bone and teeth. [63]

## **2.1.6 Lead and Health Effects**

Lead can cause a variety of health effects including acute and chronic conditions in many systems of the human body. However, these adverse effects depend on the characteristics of the exposed individual, dose, and the duration of exposure to lead. The main acute and chronic effects are discussed in the next sections. Chronic effects are discussed below in greater detail because this study involves a biomarker identifying long term lead exposure.

### **2.1.6.1 Acute effects**

Acute lead poisoning is relatively rare, and occurs when people have been exposed to large amounts of lead which is absorbed into the body over short periods of time.

Tsuchiya et al (1979) indicated that acute effects of lead toxicity are found at BLLs above

80µg/dL. Symptoms of acute lead poisoning include intestinal colic, anorexia, dyspepsia and constipation. Intestinal colic is the first symptom of acute effects, with subsequent loss of appetite, nausea, constipation and abdominal pain. [70, 75] Patients with acute lead toxicity become pale due to capillary spasms of the skin, have low pulse rates and an increase in blood pressure is common. Acute lead encephalopathy occurs at BLLs between 80 to 300µg Pb/100ml. [75] The symptoms of lead encephalopathy are disorientation, cerebral oedema, vomiting, ataxia, hyperkinesia and drowsiness. [70, 75] Acute effects of occupational exposure to inorganic lead have decreased considerably due to protective measures and occupational standards. In developed countries, it is now quite rare to encounter cases of acute lead poisoning.

### **2.1.6.2 Chronic effects**

Lead exposure leading to acute effects has gradually declined in most developed countries. However, long term low levels of exposure seem likely to continue to pose health risks. The most important chronic effects include impacts on the nervous system, and the haematological system as well as renal, reproductive effects, oral health effects, and other effects including hypertension and cancer.

Over the past thirty years, many studies have reported on the neurological effects of lead in children exposed from the environment. In the late 1970s, Needleman and colleagues (1979) reported an inverse relationship between dentine-lead levels and the neurobehavioral development of children. [76] In a follow-up study of the same cohort eleven years later, Needleman et al (1990) re-examined nearly 50 percent of young adults who had been studied in the earlier research, and reported that those who had higher dentine lead levels (>20ppm) had a significantly higher risk of failure to complete high school and lower reading ability compared with those who had dentine lead levels less than 10ppm. [77] A subsequent cohort study investigated the same participants aged 19-20 years, and found neurological deficit in those with higher childhood dentine-lead levels. [78]

A case study conducted by the Agency for Toxic Substances and Disease Registry (ATSDR) among children with low and moderate blood lead levels (10-50µg/dL) showed that lead is associated with decreased rates of growth as well as nervous and blood system

dysfunction. In adults, such exposure leads to elevated blood exposure, and nervous and reproductive dysfunction. [29] In 1991, the US Centers for Disease Control and Prevention determined that the acceptable blood lead level in children (BLL) was 10µg/dL. [79] However, evidence suggests that subtle effects can occur at even at level below 10µg/dL . [10, 30]

Evidence suggests that lead-associated intellectual deficits occur at very low blood lead levels, even below the WHO guideline value of 10µg/dL. [4, 10, 80] A systematic review of 7 and 14 cross sectional studies of tooth and blood lead levels respectively reported a doubling of BLLs from 10 to 20µg/dL or from 5 to 10µg/g tooth lead levels were associated with a deficit of intelligence quotient (IQ) of 1-2 points. [80] A more recent study by Canfield et al (2003) demonstrated that blood lead concentrations lower than the WHO and CDC guideline of 10µg/dL were inversely related to IQ. BLLs in 172 children at 6, 12, 18, 24, 36, 48, and 60 months of age were measured and intelligence tests undertaken at the ages of 3 and 5 years. Each increment of blood lead concentrations of 10µg/dL was linearly associated with a decrease of around 4.6 IQ points. [4]

The control of lead at work regulation in the UK (2002) provides the guideline of occupational exposure limit for lead in relation to an 8-hour time-weighted average working period. Concentrations of lead alkyls and other lead types in the atmosphere to which any employee is exposed of 0.10mg/m<sup>3</sup> and 0.15mg/m<sup>3</sup>. For the assessment process, BLLs are measured in these potentially exposed to lead. BLLs in women of reproductive capacity and other employees should not exceeded 20µg/dL and 35µg/dL. [81]

With regard to the haematological system, a relationship exists between environmental lead exposure and iron deficiency anaemia. Chronic lead exposure contributes to anaemia because lead interferes with haemoglobin synthesis. [75] Wright et al examined the association between iron deficiency and low-level lead poisoning of 3,650 children aged 9 to 48 months in an urban primary care clinic. Children with high blood lead levels (>10µg/dL) had a greatly increased risk of iron deficiency. [82] Subsequent cross-sectional studies have also reported a link between BLLs and iron deficiency anaemia. [83, 84] Lead is a cation substance, and its metabolism is influenced by similar factors to those that affect calcium metabolism. Thus, mineralised tissues are storage areas for lead. [85]

Concerning renal effects, previous studies have revealed that exposure to lead causes renal dysfunctions, including interstitial fibrosis, tubular atrophy, reduced glomerular filtration and nephritis. [86, 87] Recent reports indicate that lead may be nephrotoxic at lower concentrations than those formerly believed [88] with evidence that blood lead levels as low as 10µg/dL can result in renal tubular damage. [89]

Numerous studies have attempted to explain the correlation between blood lead levels and renal function including blood-urea nitrogen, serum creatinine and urine. [88, 90-92] A two-year prospective study of 121 patients with chronic renal deficiency and no history of lead exposure, showed an increase in the serum creatinine levels with increasing BLLs. [91] Similarly, an examination of data in the Third National Health and Nutrition Examination Survey (NHANES III) in the USA, by Muntner et al (2003) found that lead exposure in persons with hypertension was strongly related to elevated serum creatinine and chronic kidney disease defined by a glomerular filtration rate (GFR) less than 60mL/min estimated using the Modification of Diet in Renal Disease (MDRD) formula. [93] A cross-sectional study of occupationally exposed workers in a lead battery factory reported a strongly positive correlation between BLLs and serum creatinine as well as uric acid. [90] These two indicators are used as indicator of renal dysfunction, if the abnormal values are found assuming an increasing trend of lead exposure.

This evidence convincingly indicates that low-level environmental lead exposure may increase renal deficiency, and these effects may be exacerbated by other health conditions such as hypertension.

With regard to hypertension, long-term exposure to lead has long been known to be correlated with an increased risk of hypertension both in the general populations and among pregnant women. [94-97] In a cross-sectional study of 2,165 adult participants, Nash et al (2003) found a positive association between increased diastolic blood pressure and blood lead levels. [96] Likewise, Rahman et al (2006) investigated the effects of low-levels of exposure to lead on the blood pressure of 244 Pakistani adults, and found that an increase in systolic blood pressure was significantly predicted by increased BLLs. [98] Previous studies reported that the prevalence of hypertension is associated with occupationally exposed people living in industrial areas with lead contamination. [99]. Studying the relationship of blood and tibia lead levels with systolic and diastolic blood pressure and hypertension in a cross-sectional analysis of 964 men and women aged 50–

70 years, Martin et al (2006) found that tibia lead was only marginally associated with hypertension status after adjustment for race/ethnicity and socio-economic status. [100] However, other studies found inconsistent associations between BLL and hypertension and suggested that because lead in blood has a short half-life and indicates only recent exposure, BLL may not reflect overall exposure. [101]

Reproductive effects have been widely reported to be associated with lead exposure mainly following occupational exposure. High blood lead levels may result in abortion and pre-term delivery in pregnant women, and can decrease fertility in men. [63] Borja-Aburto et al (1999) reported that low to moderate levels of lead exposure may increase the risk of spontaneous abortion. A case-control study of 668 pregnant women in Mexico City was conducted using interview data and blood lead levels. Spontaneous abortions before week 21 (n=35) were matched with pregnancies that survived beyond week 20 (n=60) for maternal age, hospital, date of enrollment, and gestational age at enrolment. Mean BLLs were significantly different, and the odds ratio for spontaneous abortion was 1.8 (95% CI=1.1, 3.1) for every 5µg/dL increase in blood lead levels. [102] Some animal studies also support these findings. [103] Lead has also been associated with low birth weight and other reproductive effects. [104] An inverse relationship has been found between dietary calcium uptake and lead absorption, implying that children with calcium-deficiency seem likely to absorb greater levels of lead than calcium-sufficient children. [53]

Cancer has also been reported to be associated with lead exposure. The International Agency for Research on Cancer (IARC) has identified lead and inorganic lead components as Group 2B: probable human carcinogens, however these conclusions are based on animal studies which mostly used high doses of lead. [53] Current evidence of the carcinogenicity of lead in humans remains inconclusive. [105, 106] Studies of the relationship between occupational lead exposure and cancer are often limited due to confounding exposures such as other chemical exposure (arsenic, cadmium) and smoking. To explore the relationship between lead toxicity and stomach, brain and lung cancer, meta-analyses of epidemiological studies have been conducted, which controlled for confounding factors. No consistent association was found between environmental lead contamination and cancer. [107-109]

Although epidemiological studies do not convincingly demonstrate the carcinogenicity of lead, some experiments on animals do provide evidence that lead exposure increases the risk of tumorigenesis. The proposed mechanisms of lead carcinogenicity involve DNA damage, clastogenicity and the inhibition of DNA synthesis or repair, as well as changed gene expression. [110]

The influence of oral health have been demonstrated. Several studies have examined the relationship between environmental lead exposure and dental caries. [111-113] An early study undertaken by Curzon (1983) looked at the prevalence of caries in populations having high lead levels in both soil and water compared with control groups. Children residing in high-lead environments presented a 40% greater prevalence of caries. [111] In addition, in the US Third National Health and Nutrition Examination Survey (NHANES III), a relationship between BLL and the risk of tooth caries was reported. An increase in BLL of 5µg/dL was correlated with approximately twice the risk of dental caries. [114]

### **2.1.7 Global distribution for human exposure to lead**

The prevalence of lead exposure and exposure levels vary between countries. In most developed countries there has been a steady reduction of BLLs due to a decrease in lead use. Nevertheless, high BLLs in children are still found in most developing countries. Low-income countries account for the highest BLLs and largest lead body burden. [115] In 2004, WHO revised the global burden of disease and found 16% of children worldwide had  $BLL > 10\mu\text{g/dL}$ . Of all children who had elevated BLLs, almost 90% lived in the low-income areas. [115] A longitudinal study reported that in Bangkok, Thailand, mean BLLs in 564 primary school children were  $9.3 \pm 3.7\mu\text{g/dL}$ , although 27.4% of the children had BLLs higher than  $10\mu\text{g/dL}$ . [34] Another study of primary school children in Jakarta, Indonesia, showed a mean BLL of  $8.6\mu\text{g/dL}$ , where 35% of the children had BLL greater than  $10\mu\text{g/dL}$ . [33] Such reports indicate that high BLLs still occur in middle and low-income countries.

In addition, highest BLLs were associated with plumbosolvent water. This study is similar to other epidemiological studies found that geographical, environmental and personal factors influence BLLs in people.

There remain some of the world's most polluted areas in the developing world in La Oroya, a mining town in Peru. A metal smelter has operated for more than ninety years. Since 1922, people in this area have been exposed to polluted emissions and waste. Metal smelting and toxic emissions from mining and processing operations have resulted in high lead levels potentially affecting 35,000 people living around this town. According to a survey undertaken by the Peruvian Ministry of Health in 1999, the average BLL in children aged 6 months to 10 years was 33.6µg/dL, over three times higher than the WHO guideline of 10µg/dL. This finding indicated that children are being exposed to high levels of lead and the neurological effects of this are a serious concern. Other pollutants such as sulphur dioxide also exceed the WHO limit, and contamination by other metals including lead, arsenic and cadmium are likely to compound the situation. Environmental management plans for the processing plant, including site remediation and emission control systems, have been proposed since 2004, but not yet implemented. The local authorities implement blood screening and testing for vulnerable people, especially children and pregnant women in order to monitor BLLs in the region, but additional pollution control procedures and public health programmes are urgently required in this highly-polluted area. [8, 116]

Port Pirie, a mining town in Australia is another example of a high pollution area and one which provided much insight into the association between environmental lead and neuropsychological development in early childhood. Over 600 children born between 1979 and 1982 were recruited into a cohort study, BLLs were collected and measured during post natal periods at 6, 15 and 24 months of ages. The results indicated an association between blood lead levels and mental development at 2 years of age. [117]

### **2.1.8 Factors influencing human lead exposure**

There is a substantial amount of evidence to suggest that lead exposure in humans is associated with various factors including demographic, environmental and socio-economic variables. [33, 118-120] This section provides information concerning the different factors which influence human lead exposure, and which were explored in the Tooth Fairy Study.



### 2.1.8.1 Demographic factors

Several studies suggest that BLLs are related to age. [5, 121, 122] A cross-sectional study to determine mean blood lead levels and their sociodemographic correlates in 13,201 American children aged 1 year and older examined during the first phase of the National Health and Nutrition Examination survey (1988 to 1991) reported that younger children aged between 1 and 5 years had higher BLLs than older children. [123] BLLs are thought to be higher in younger children at around 1-2 years old due to their hand to mouth behaviour. [79, 124]

Some studies have reported sex differences in BLLs. Yapici et al (2006) studied 236 children aged between 6 months and 6 years, living around a coal-mining area in Turkey. [7] Mean BLLs in boys ( $38.8 \pm 16.0 \mu\text{g/dL}$ ) were significantly higher than those in girls ( $33.8 \pm 15.6 \mu\text{g/dL}$ ) ( $p < 0.05$ ). In addition, a study of the determinants of BLLs over  $10 \mu\text{g/dL}$  in primary school children in Bangkok reported an odds ratio of 1.86 for males compared to females. [23] However, a report of lead levels in 143 permanent teeth in non-occupationally exposed people aged 14-60 years in Israel showed no statistically significant difference between tooth lead levels in men and women. [125] This finding supports research conducted in Australia and Taiwan. [126, 127] A possible explanation for such differences findings is that lead dose is not only determined by sex, but also physiological and lifestyle factors in children and adults.[128]

Lead levels can differ between people of different ethnicities living in similar environments. A study of adults between 50 and 70 years in Maryland, USA ( $n=1,140$ ) suggested that lead levels in the tibia bone of African Americans were 29% higher than those in European Americans. [129] In addition, a survey evaluating BLLs in children (aged 1-5 years) at risk of lead poisoning during a 16-year period in the United States, found that a high BLL is a risk factor of lead exposure in non-Hispanic black people compared to non-Hispanic white people. It seems probable these results are due to differences in socio-economic circumstances, such as living in old residences which may contain lead paint and/or water pipes. [130]

### 2.1.8.2 Socio-economic and occupational factors

A relationship exists between socio-economic factors, such as family income, and lead exposure. [122] In UK, a survey of BLLs carried out in 8,500 people found that BLLs were significantly associated with social class especially in children aged 2- 4 years old. [131] An analysis of data from the Third National Health and Nutrition Examination survey between 1988 and 1994 examined 3,325 Mexican-American children aged 1 to 17 years. The poorest children were 5 times more likely to have a BLL greater than 10µg/dL compared to the wealthiest group. [122] One possible explanation for this finding is that more low-income families live in older homes with lead paint. In addition, malnutrition leads to an increasing of lead absorption in those poor people. [132] Other studies have also shown associations between human lead levels and levels of education and socioeconomic status. [133, 134]

Parental education is another indicators of socio economic status, has also been shown to associate with lead exposure. [135] A study of lead poisoning among African and Arab American children found that elevated BLLs are associated with low educational levels of their parents. [132]

Occupational lead exposure is known to be the major source of lead poisoning in adults, but occupational lead exposure is also hazardous to individuals residing with the exposed worker. [136] Occupational lead exposure still occurs in developed countries, but is a particular risk in developing countries. [7, 137] In cottage industry settings especially occupational exposure also impacts on children in the household. An investigation of lead exposure in household members exposed to battery repair environments in Jamaica reported BLLs $\geq$ 25µg/dL in all age groups, where 43% of the children aged less than 12 years had BLLs exceeding 70µg/dL. It seems possible that high BLLs are due to high concentrations of contaminated lead in soil and dust on exposed surface. [138] Other researchers have identified similar trends, where living or working in areas exposed to high lead concentrations, such as shipyards, mines, or radiator repair, results in elevated human lead levels. [139, 140]

Previous studies have demonstrated a relationship between high dental lead levels and occupations involving the use of lead. One cross-sectional study showed that a major risk

factor for high enamel lead levels is having a household member work in the manufacturing of paints, paint pigments, ceramics and batteries. Household members or parents can bring home lead-dust contamination via their skin, hair, clothes and shoes. [141] It has also been reported that car repair activities in homes, yards and gardens may be associated with the contamination of soil with lead. [142]

### **2.1.8.3 Hand to mouth behaviour and soil**

A possible pathway for exposure to lead in children is the ingestion of lead from soil via hand to mouth behaviour. Excessive mouthing behaviour, for which the term 'pica' has been introduced, is an abnormal behaviour involving eating something that is not normally eaten. Children who practice pica are likely to ingest non-food substances such as clay, dirt, sand, stones, paint chips, coal, wood or plaster. A previous study in 1976 has suggested that around 50% of young children aged 1-3 year old exhibited such behaviours. [143] A longitudinal study of dentine lead levels, intelligence, school performance and behaviour in children found that children with a history of pica had higher DLLs than those who did not. [133] In addition, children who engage in pica in association with contaminated soil or house with lead containing paint might be expected to receive considerable lead exposure resulting in elevated BLLs. [144]

Several studies have focused on soil as a major factor in child's exposure to lead. A survey of soil contamination in domestic gardens and allotments in 50 cities across England, Scotland and Wales concluded that lead, cadmium, zinc and copper were the most significant hazards in garden soils. [145] Urban soils have contaminant enrichment. [146-148] The major sources of lead pollution in urban soil are vehicle exhaust emissions and paint containing high levels of lead. [46] Regarding soil lead exposure in the present study, Newcastle is located on the river Tyne and has been a highly industrialised city in the past. Heavy metals including lead in soils and allotments were found, [149] results from a study particularly on soil lead levels were used to discuss possible pathways of children lead exposure in the Tooth Fairy study. [149]

In the UK, soil guideline values (SGV) for lead as a function of land use are defined by the Environment Agency [150]. The SGV for residential areas with or without plant uptake as well as in allotments is 450mg/kg while that for commercial and industrial land use is higher, at up to 750mg/kg. Based on a study of soil lead levels taken from 163

samples in the Byker area of Newcastle, the median soil lead was 234.5mg/kg (mean±SD=350±472mg/kg). It was noted that the maximum value of lead concentration in soil in the Byker study, 4,134mg/kg was far higher than the relevant SGV, and 37% of the samples taken contained soil lead levels ranging between 443-615mg/kg. [151]

#### **2.1.8.4 Residential areas and house conditions**

Several studies have found higher lead levels in those living in older houses where lead-based paint remains an important source of lead exposure. [152] [79] Although regulations limiting lead in paint have resulted in decreasing levels of lead being found, lead paintwork in houses remains often in deteriorating condition meaning exposure to lead is still possible. [153]

Land use near to and within residential areas is a potential source of lead exposure. Mean teeth lead values in Brazilian children living in an industrial area were significantly higher than those in non-industrial areas at 169µg/g and 118µg/g respectively. [154] In the Czech Republic, children living in areas with heavily contaminated soil had high tooth lead levels (6.44µg/g) compared to those living in the less contaminated area (1.45µg/g). [155] Mean lead levels in deciduous teeth of Egyptians in urban and rural areas were  $7.96 \pm 5.20\mu\text{g/g}$  and  $4.97 \pm 3.77\mu\text{g/g}$ , respectively. [156]

House dust has been studied widely as a potential source of lead exposure, especially to young children. [157] It is known that activities in houses such as the disruption of surfaces, window replacement, demolition and renovation, are likely to generate elevated lead dust on the house's floors and indoor surfaces. [158] If these houses are not cleaned properly, amounts of lead dust will remain on carpets or windows, doors, stairs and furniture.

#### **2.1.8.5 Dietary intake**

Most lead ingested into the human body comes from food. Contamination drinking water has also been found to be associated with lead dose especially where drinking water is supplied using lead pipes or lead soldered joints. [159] In the UK, lead contamination from drinking water pipes has been found especially in the houses built before 1930s

which usually have lead water pipes. [153] The water supplies to the houses usually pass through the main pipe lines, and smaller lines subsequently deliver the water from the main line to each house. [160] Over several decades, lead pipe lines have been replaced during renovation. During such renovation, high lead levels in tap water were found. One study noted that lead leaching from the surface of lead lines increased when changing from chlorine to chloramines, disinfectant products resulting in higher lead levels in tap water. [161]

Bottle-fed infants may suffer substantial lead exposure if the water used to make up their formula is contaminated by lead. A study in the Netherlands indicated that bottle-fed infants are at risk of lead exposure, especially if fed formula in the first six-months. [162]

The association between lead and dietary calcium has been studied widely in animals and humans. [163-165] Calcium is a major nutrient in milk and dairy products, and calcium sufficiency protects absorption against lead. Insufficient dietary calcium can increase lead absorption in human. Consistent with this, the CDC found that children with elevated BLLs are at greater risk of poor nutrition. [152] However, some observations suggest an inverse relationship between higher calcium intake and greater levels of lead exposure.

Iron is another micronutrient found to affect metal toxicity. Iron interacts with heavy metals *in vivo* leading to changes in how they are metabolised and transported. Little is known about the interaction between lead exposure and nutrient status, but it seems likely that iron deficiency increases lead susceptibility via the greater absorption of lead. [82, 164] Iron deficiency is a malnutrition problem found worldwide. As a result, a substantial number of studies have been conducted concerning the public health problems of lead toxicity in iron deficient people. [82, 164]

#### **2.1.8.6 Health**

Health conditions documented to be related to lead exposure include asthma and eczema disease. Although the pathological mechanisms linking asthma and lead are not clear, higher blood lead concentrations may be associated with excessive immunoglobulin E which possible increasing risk of asthma. [166] Some American studies have suggested elevated lead levels and increased risk of asthma are more commonly observed in low-income children indicating that these vulnerable groups are more susceptible to heavy

metal exposure and its impacts. Another health condition linked to lead exposure is eczema, a common skin disease, the incidence of which has been increasing in developed countries, [167] with environmental, hygiene and dietary factors being associated with its occurrence. A previous study has reported that serum lead levels were positively correlated with eczema severity. [168]

BLL is commonly used to determine the health effect. The CDC has recommended a blood-lead level of 10 µg/dl in children as acceptable, but levels above this require preventive intervention. According to the CDC recommendations, for example, if children have blood lead levels between 10 and 14µg/dL, blood lead testing should be repeated every 3 months. In addition, advice on reducing lead exposure should be provided to parents. For children who have BLLs of 15 to 19µg/dL, blood lead testing should be repeated every 2 months, and potential lead sources as well as histories of lead exposure should be considered. Parents should also be given guidance about interventions to decrease BLLs, such as environmental lead reduction and nutritional intervention. Moreover in children with  $BLLs \geq 20\mu g/dL$ , individualized case assessment should be considered, such as physical and nutritional assessment, the investigation of environmental lead exposure and medical histories. Medical intervention such as chelation therapy may be required in severe cases. [135]

#### **2.1.8.7 Other factors**

Smoking is another potential source of lead exposure. Exposure to lead may occur through inhalation of lead contaminated smoke particles as well as by often hand to mouth contact especially in an occupational setting where lead exposure exists. [169, 170]

Several other factors have been reported to influence lead levels in humans include eye cosmetic products and leaded ceramics. [171-175] One such cosmetic is Kohl, also known as kajal, al-kahl or surma, used as makeup for the eyes, predominantly by women in Asia, the Middle East and most areas in Africa except for South Africa. [172, 176, 177] Kohl is a powder containing a mixture of antimony and lead sulphide. Since antimony is expensive, lead sulphide has replaced antimony in manufacture. For followers of the Islamic religion, kohl was applied to infants until recently. One comparative study

revealed that infants (6-16 months old) to whom kohl was applied had significantly higher BLLs than infants without this exposure. [178]

In the UK, the first report of lead poisoning from eye cosmetic use in a 3-year-old Indian boy, this child was admitted to hospital with lead encephalopathy, and a BLL of 178µg/dL was identified. X-ray analysis indicated lead in the long bones and intestinal opacities, suggesting ingested lead. His mother and 5 years old sibling also had high BLLs of 65µg/dL and 72µg/dL, respectively. [179] An investigation of the housing conditions was unproductive since they had recently moved. A possible pathway for lead exposure was the mascara-like substance applied to their eyelids. A sample of the powder from India was found to contain 80% lead sulphide. Following this serious case several reports of lead poisoning from this cosmetic in many areas, in particular in Asia and the Middle East, have been tested. Although kohl is more commonly used in certain areas, it is also available in the USA and Britain, implying that the use of this hazardous substance is not limited to developing countries. It would be worth promoting the use of lead-free kohl in order to ensure lower exposure to lead. [178]

Lead glazes are most commonly used on ceramics and they produce attractive glazes. When use glazes are properly formulated and fired to sufficient temperature, the lead is sealed. Nevertheless, if these products are not prepared properly, lead can leach into food in contact with on the ceramic ware. The degree of leaching varies depending on how often tableware or ceramics are warmed and used. In addition, food containing acidic ingredients is more likely to be affected. [180]

## **2.2 Biological monitoring of trace elements**

### **2.2.1 Introduction**

Biological markers (biomarkers) are cellular, biochemical, and molecular alterations which are measured in biological compartments such as human tissues, cell or fluids. [181] Biomarkers commonly include biological characteristics that can be assessed and measured as bioindicators of biological, pathogenic and pharmacological processes following therapeutic intervention, occupational and environmental exposure. [182, 183]

Biomarkers can be defined according to their role into three classes: biomarkers of exposure/dose, effect (or response) and susceptibility. Biomarkers of exposure are primarily indicative of the dose or amount of toxin circulating systemically or within a specific compartment of the body. These biomarkers can reflect short or long-term exposure or dose depending on the toxin, media and metabolism. Biomarkers of effect or response measure the biological changes in an organ or system as a result of exposure to a specific toxin. Lastly, biomarkers of susceptibility indicate an individual susceptibility to toxicity. These involve relevant factors such as acquired ability, or genetic markers that reflect susceptibility to toxins. [184]

Biological monitoring refers to the systematic measurement and assessment of agents and metabolites in either tissues or secretions in order to assess the uptake of chemicals and better assess risks to human health. [185]

The main purpose of the biological monitoring of metal toxins include the measurement of current and past levels of exposure as a body burden, determine both individual and group risk, provide data on dose-response relationships, and assess the potential benefits of medical treatment. [185] Biological monitoring requires suitable media which can be collected and analysed in order to provide this information.

## **2.2.2 Biomarkers for monitoring lead exposure**

Biomarkers used to assess human lead exposure include measures of lead in blood, hair, saliva, nail, bone and tooth. Each marker has strengths and weaknesses depending on the propose of use. [5] This section provides a review of various biomarkers for environmental and occupational lead exposure which have been reported in the literature.

### **2.2.2.1 Blood and plasma**

Whole blood lead level is the primary biomarker most commonly used to determine human lead exposure. [5, 29] Most epidemiological studies have used BLLs as the biological marker of exposure. Several studies have explored the association between BLLs and different health effects, including deficits in IQ, growth development, renal function, hypertension and oral health [91, 186, 187], as discussed in Section 2.1.6.



Around 95% of lead in blood is found in the erythrocytes (bound to haemoglobin), where it is in dynamic equilibrium with lead in plasma. [188] Although BLL is the current standard biomarker, lead in blood has a short half-life of around 28-36 days [189], and evidence suggests about 20 days in children [14, 190] and 40 days in adults. [14] The half-life of lead in plasma is even shorter, at 1 hour. [191] Lead in plasma is in equilibrium with extracellular compartments and is therefore an indicator of lead levels in soft tissues. However due to its low lead levels, plasma as a biomarker to identify lead exposure is of limited application using currently available analytical methods. The relationship between blood lead and plasma lead is complicated. There is a linear association at BLLs lower than 40µg/dL; however, a curvilinear relationship has been found above this level. [192]

Even though BLLs are commonly used, there are limitations to this biomarker particularly as a measure of long-term exposure. Due to its short half-life, a measurement of blood lead can only provide an indicator of recent exposure i.e over preceding months. In addition, the relationship between BLLs and other metabolic effects such as heme and nucleotide synthesis is nonlinear. [71] Moreover, there is a reversible exchange of lead between blood, bone and soft tissues, so that a single measurement of blood lead cannot distinguish low levels of long term exposure or short-lived high levels of exposure. To overcome this limitation, some studies suggest the determination of serial-blood lead, where data can be used to calculate an average BLL over a lifetime, named the 'lifetime average integrated blood lead level'. [189] While this approach might be useful in assessing occupational exposure, they are obvious practical and ethical issues in applying such as approach in large scale epidemiological studies.

#### **2.2.2.2 Urine and Faeces**

Due to the excretion of lead in urine, urinary lead levels provide a noninvasive biomarker of lead exposure, although urinary lead is normally considered less reliable than BLL for the measurement of lead exposure. [71] Urine lead levels are not a good proxy for BLLs (the gold standard) because they are not correlated at low lead levels, [193] and using urine as a marker is potentially contaminated during sampling process.

Despite the limited use of urinary lead levels for the biological monitoring of environmental lead exposure, the chelatable lead excreted in urine is regarded as a crucial marker of lead toxicity since it is known to be significantly associated with the symptoms of lead poisoning. [71] After the administration of chelating agents such as calcium disodium ethylenediamine tetraacetic acid into the human body, levels of chelating lead can be determined within 24 hours. [71] Other studies have explored various metabolites or substances in urine in response to lead exposure, such as microglobulin and coproporphyrin, but these methods are not specific to lead and their reliability as clinically relevant markers is not clear. [194]

A study in young children found a significant relationship between lead paint and faecal lead excretion. [195] In addition, this non-invasive biomarker can be used to assess lead concentrations in animals. However, using faecal lead levels may be biased by types of food intake and behaviour. [196] Therefore, faecal lead is not widely used in current research.

### **2.2.2.3 Saliva**

Saliva is also easy to collect and can be used to monitor lead exposure [5, 197], although there are concerns about the validity of this biomarkers. Within the oral cavity, high lead concentrations influence the salivary secretion of protein, calcium and enzyme, but the ion composition of also saliva changes during the day, particularly at meal times. High lead concentrations may therefore influence neurological and emotional status, changing saliva quality and quantity. [197] Although these changes may also occur independent of lead exposure, therefore, there is a need to clarify the value of using saliva to measure lead exposure.

In addition to the issues noted above, regarding interpreting salivary lead levels, the half-life of lead in saliva is known to be shorter than that in blood. [198] One study found a rapid decrease of salivary lead concentrations in 5 male workers occupationally exposed to lead, suggesting half-life of lead in saliva of 5-7days. Cleymaet et al also examined salivary lead concentrations over a very short period, and found a short half-life of only 1 hour. [199]

The evidence suggests that the validity of using saliva to determine environmental lead exposure could be confounded especially by metallic dental restoration and intra-oral orthodontic equipment. [200] Therefore, it has been suggested that salivary lead measurement is of limited use for assessing chronic low-level exposure.

#### **2.2.2.4 Sweat**

Skin lead absorption is a key route of exposure in occupational setting such as lead battery factories or smelting workplaces, [90, 201, 202] and sweat is a particularly important route for eliminating dermally absorbed trace metals, including organic lead, from the body. [202-207]

Omokhodion et al measured levels of lead in the sweat, blood, urine and saliva of 24 healthy men All subjects were thermally induced to sweat by sitting in a hot chamber at 40-45°C, with 35% relative humidity, for 1 hour. Sweat was collected using the arm bag method. Mean lead levels were in the order of blood>urine>sweat>saliva. There was a significant relationship between blood and sweat lead levels ( $r=0.7208$ ). On the other hand, only weak correlations between blood and urinary lead levels ( $r=0.234$ ), and blood and saliva ( $r=-0.1864$ ) were reported. [207] These findings disagreed with those of Omokhodion and Crockford who applied lead chloride orally to two adults. Sweating was induced by cycling in a hot chamber, and sweat was collected in polythene arm bags. Although there were marked increased lead levels in the blood and urine, no increase in lead levels in sweat was detected. [206]

Results from such studies indicate that lead levels in sweat can be used as a biomarker to indicate exposure to lead. Lead levels in sweat are more useful in detecting acute dermal exposure to lead, where blood lead levels may be unreliable.

#### **2.2.2.5 Hair**

Lead has been widely measured in hair with the purpose of detecting excessive levels of exposure from environmental contamination. [208] Hair lead levels have been showed to correlate with levels in blood and bone. [209, 210] Many researchers have reported the advantages of measuring levels of lead in hair. It is convenient and non-invasive to

collect, and it is easily transported and stored for later analysis. [211] Moreover, retention times of lead in hair has been determined to be between 3 and 5 months, [210, 212] suggesting that lead levels in scalp hair may reflect longer periods of exposure than those in blood. [213] In a Russian study of 189 kindergartens children, mean blood lead levels ( $9.8\mu\text{g/dL}$ ) were higher than hair lead levels ( $7.2\mu\text{g/g}$ ). Moreover, the authors concluded that measuring hair lead levels was not a suitable method for screening children for lead poisoning owing to low sensitivity (57%), and a high degree of false-negatives (18%). [214] A drawback of this approach is that the washing of hair samples during preparation which may cause lead concentrations to fall. [215] Additionally, different washing methods may affect lead levels differently, so results obtained using different approaches are difficult to compare. [215]

Hair lead levels vary significantly within each individual. A considerable volume of literature has been published which reports correlations between blood and hair lead levels. [216] Despite these possible limitations, hair lead concentrations have been used in various epidemiological studies. [217-220] Several factors have been found to influence hair lead levels, including age, sex, smoking, hair cosmetics, place of residence, and adult occupational exposure. [211, 217, 221-223] Some studies suggest that hair lead concentrations are higher in people living in exposed areas such as intensive traffic, urban, industrial areas than those from less contaminated areas or rural zones. [212] Recent evidence suggests that occupational exposure such as among workers in automotive or lead refining plants are more likely to have higher hair lead levels than others. [220, 224]

Lead has been found in many hair cosmetics such as hair treatment and henna [225, 226], and the long term use of these products possibly increases exposure to lead. For example, a study in Kuwait evaluated lead levels in henna, a reddish-brown substance used to change the colour of hair. Low lead concentrations at between 2.29 and 65.98 ppm were found in twelve commercial hennas. [225] The absorption of lead from hair colouring agents may represent around 0.5% of the total absorption from the average daily environmental lead intake [227] meaning long term users of such products may be exposed to low lead exposure.

It appears that hair lead measurement as indicator of internal dose may be of limited use due to external contamination and the impact of sample preparation techniques limit

comparison between studies. In general, therefore, it is unlikely that hair lead levels could be suitable biomarker for cumulative exposure.

#### **2.2.2.6 Nail**

Fingernails and toenails are easily collected, non-invasive biomarkers for metal analysis. Numerous studies have reported the use of nails to assess environmental lead exposure. [228-231] Nails can indicate long term lead exposure, and are generally stable and a constant component after collection. Because toenails grow up to 50% slower than fingernails, they may be more useful for the determination of longer term lead exposure. [5, 228] Rashed and Hossem (2007) measured lead levels in the toenails and hair of children, adults and workers living in polluted and unpolluted areas in Egypt. Lead levels in the fingernails and hair of children living in polluted areas were significantly higher than in those residing in unpolluted areas. [232] Unfortunately, another study by Gulson (1996a) suggested that nails may be of limited use as a biomarker due to large variation in lead levels in the same fingernail and toenail samples. [228] Overall, it appears that the use of nail lead levels to identify environmental lead exposure must be carefully considered.

#### **2.2.2.7 Bone**

Tsuchiya reported that around 90% of absorbed lead is deposited in the skeleton. [70] Therefore, the identification of lead in bone may be suitable for evaluating the body burden of lead. Bone lead levels are considered to be a reliable bio-marker for long term exposure with a long half life of between 10 and 30 years. [127] However this matrix requires a bone biopsy which is invasive and not suitable for epidemiological studies. [5] Over the last decade there has been increasing acceptance that in vivo bone lead may be assessed by using x-ray fluorescence. However, lead levels vary in different bone types, due to the rate blood is delivered to each type of bone. [233] Trabecular and cortical bone have different rates of turnover and thus the measurement of bone lead should be performed separately. The cortical bone of the tibia and phalanx, and the trabecular bone of the patella and calcaneus are most commonly used to measure lead exposure in epidemiological studies. [234] A long term cohort study measured lead concentrations in dentine, blood and bone (tibia and patella), and compared the measurement of these

matrices with neuropsychological test scores in 79 teenagers aged 19-20 years. The results found that DLLs were inversely associated with those test scores. [78] Another cohort study used the same participants found that DLLs were associated with body mass index (BMI), while lead levels in bone were not associated with BMI. The authors concluded that the use of X-ray fluorescence had limited precision especially in determining for bone-lead determination. [235] Overall, levels of lead in bone can be used to identify long-term lead exposure. However, a suitable technique to examine bone-lead levels must be chosen.

### **2.2.2.8 Teeth**

Teeth have more recently been recognized as a potential medium for determining long term lead exposure [1, 5, 236, 237] because it is known that the half-life of lead in calcified tissues, including permanent teeth, is around 10-20 years. [70, 71] Unlike bone, the mineral phase of tooth development is quite stable. [238] Furthermore, the exfoliation of primary teeth starts at age 6 and exfoliated teeth very easy to collect. [5, 239]

As a result, levels of lead in deciduous/primary teeth may reflect exposure throughout early life which cannot be captured using blood lead levels. Teeth can be used to assess environmental pollution because trace elements are incorporated during the mineralising phase of tooth development. [5, 240] Barbosa et al (2005) mentioned that various areas of tooth sections incorporate lead at different phases of development. [5] Deciduous teeth are special biological tissues in which prenatal as well as early postnatal environments leave traces. Therefore, the spatial distribution of trace elements in deciduous teeth can enable the determination of the exposure to pollutants over time. [13]

Previous studies have reported the use of lead in teeth as a biomarker of past exposure of children. These studies have most often analyzed whole teeth, which have been digested in acids. [133] Because we know that different components of human teeth incorporate lead differently during mineralisation, analyses of whole teeth do not allow the time specific distribution of this metal to be assessed. [13] There is therefore increased interest in studying lead exposure in different parts of the tooth to enable the effects of mineralisation over time to be assessed. Different parts of the tooth have been studied for lead exposure and have identified different levels of lead. [241] Gulson (1996) has previously determined lead exposure in human deciduous teeth and suggested that enamel

is better in determining prenatal exposure, while dentine is suitable to measure of postnatal childhood exposure. [242]

Although the use of tooth lead levels as a biomarker of long-term exposure has made considerable progress, some aspects remain unexplored. It is known that teeth develop over a chronologically specific period and that primary human teeth are formed in a well-defined incremental manner. [243, 244] Therefore the measurement of lead levels in whole teeth and fragmented enamel and dentine may not provide reliable data concerning specific periods of early life exposure because the techniques used such as digestion destroys the sections and incremental lines have lost. [133, 237] It then was not able to use these lines in measuring the ages at specific points such as in enamel which have been suggested by Humphrey and colleagues. [245, 246] The present study aims to develop histological techniques to determine patterns of lead exposure during the early period of a child's life.

A summary of lead half-life in different biomarkers is given in Table 2.2, although it should be noted that the biological half-life of lead is difficult to define [247]. Since previous studies have obtained data using different approaches and samples.

**Table 2.2** Summary of the half-life of lead in each biomarker

<b>Biomarker</b>	<b>Half-life</b>	<b>Authors [Ref]</b>
Blood and plasma	40 days (adults males)	Rabinowitz, 1976 [14]
	27 days	Needleman, 1972 [248]
Urine and faeces	Weeks	Hu, 1995 [72]
Saliva	Days	Brodeur, 1983 [198]
Sweat	Days	Omokhodion, 1991a [206]
Hair	Months	Hu, 1995 [72]
Nail	Months	Hu, 1995 [72]
Bone	10-20 years	Rabinowitz, 1991 [127] Needleman, 1972 [248] ATSDR, 2007b [249]
Tooth	≥10 years depending on deciduous or permanent tooth	Barbosa, 2005 [5]

## **2.3 Tooth morphology, development and histology**

### **2.3.1 Tooth development (Odontogenesis)**

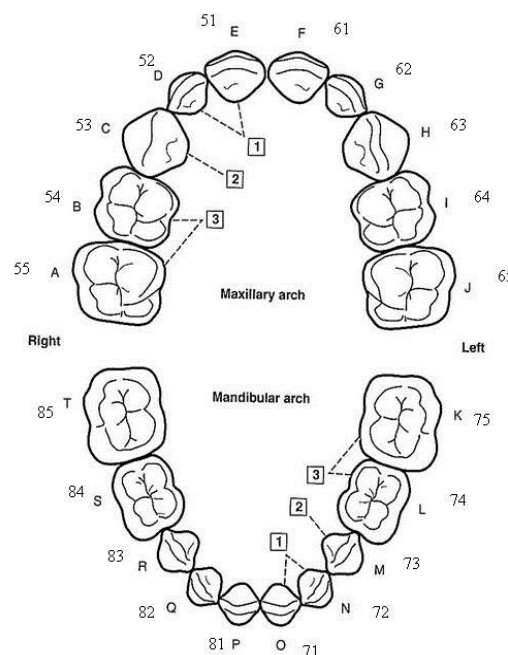
Two dentitions occur in a human's life. The deciduous or primary dentition appears between six months and three years after birth. The permanent or secondary teeth then erupt between six and twenty years of age. From the ages of six to twelve years, the mixed dentition of both deciduous and permanent teeth is present. [244, 250] Exfoliation is the process of loss of the deciduous teeth prior to their replacement by permanent teeth, known as eruption which begins about age six. Deciduous teeth begin to exfoliate around 5-6 years of age. The primary dentition remains until a child is about 6 years of age, when the transition to the permanent dentition begins. Eruption of the permanent dentition starts with incisors at 6-8 years, canines at 9-12 years, and molars at 6-12 years; however, the primary molars are replaced by permanent premolars at aged 6 years, while the durations of the eruption of permanent molar are between 6 and 12 years. The deciduous dentition includes incisors, canines, and molars, and the permanent dentition includes incisors, canines, premolars and molars. [239, 244, 250]

The two principal anatomical parts of each tooth are the crown and the root. The crown is visible in the mouth after tooth development and consists of coronal dentine, the pulp, and is covered with enamel. The interior portion of a tooth is mainly composed of dentine. Based on region, coronal dentine comprises mantle dentine and circumpulpal dentine. The circumpulpal dentine is comprised of globular dentine, intertubular dentine, peritubular or intratubular dentine, and sclerotic dentine. More details regarding each tooth region are discussed in the dentine mineralisation section. The root is located under the gum and consists of the root dentine, the root canal, a continuation of the pulp and is covered with cementum. The number of roots varies according to tooth type. In deciduous teeth, for example, incisors usually have a single root, canines normally also have one long root. Deciduous molars are different from those primary tooth types. The upper and lower deciduous molars have three and two roots respectively. [239, 244, 250] The primary dentition is shown in Figure 2.4 and the components and structures of the tooth are shown in Figure 2.5.



The Fédération Dentaire Internationale (FDI), the leading international organisation of the dental profession, provides the standard notation or code for teeth in a two-digit system. [244] The first digit indicates the quadrant and dentition, and the second digit the specific tooth. The FDI labelling systems of deciduous molars (teeth sampled in the histological study) can be distinguished as follows:

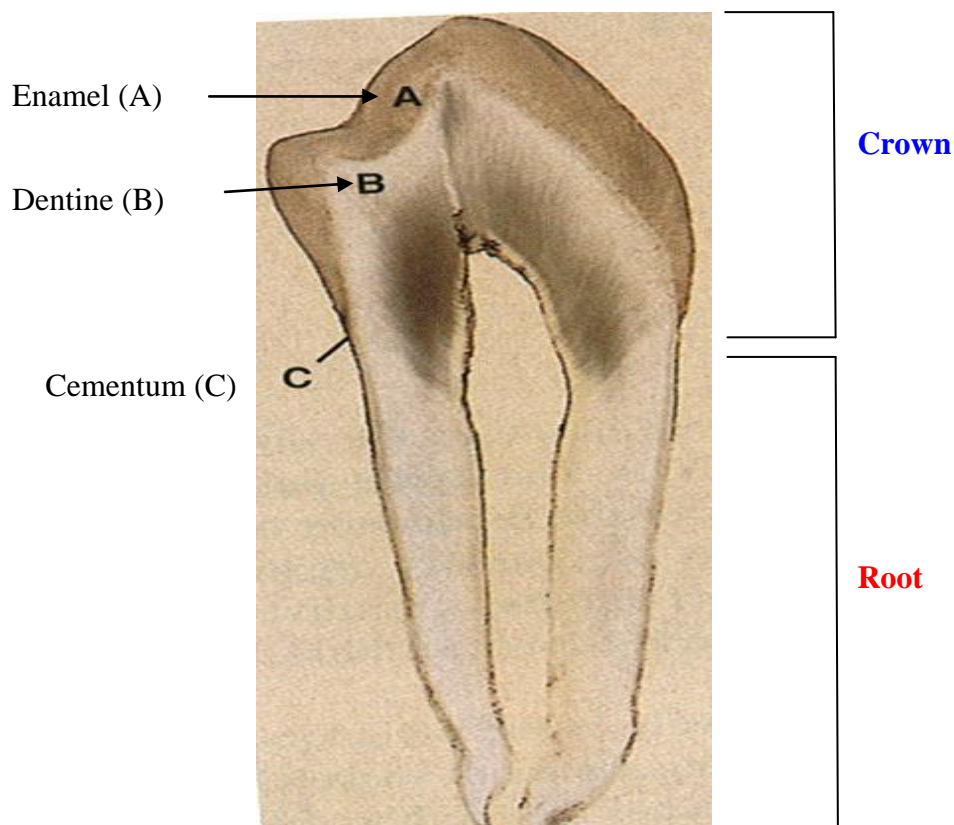
- 54 = Maxillary right first molar
- 55 = Maxillary right second molar
- 64 = Maxillary left first molar
- 65 = Maxillary left second molar
- 74 = Mandibular left first molar
- 75 = Mandibular left second molar
- 84 = Mandibular right first molar
- 85 = Mandibular right second molar



**Figure 2.4** Primary dentitions (occlusal views)

From Schroeder, HE. (1991) *Oral Structural Biology*, Stuttgart: Georg Thieme Verlag. [251]

Teeth develop over a chronologically specific period, and a time line for each tooth can be identified. [252] Tooth development begins prenatally with the first deciduous incisor at between 14 and 16 weeks after fertilization, followed two weeks later by the lateral incisor and, after another week, the deciduous canine. Deciduous first molars begin to develop 15 weeks after fertilization, followed by the second molar three to four weeks after that. The permanent incisors begin to form at three to four months after birth, followed by the canine one month later, and the upper lateral incisors at the end of the first year after birth. The premolars and second molars initiate between two and three years of age while the first permanent molar starts to develop in utero, at 28 to 32 weeks after fertilization. The initial formation of the third molar begins between the ages of seven and ten. [244] In the present study, exfoliated deciduous incisors were collected for the Tooth Fairy Study to assess determinants of lead exposure, and deciduous molars extracted for oral hygiene purposes were used to develop the histological technique to assess long term lead exposure.



**Figure 2.5** Component and structure of the tooth

From Berkovitz, BKB., Holland, GR., Moxham, BJ. (2009) *Oral anatomy, histology and embryology*. 4<sup>th</sup>ed, Edinburgh: Mosby. [239]

## **2.3.2 Dental histology**

The tooth crown is comprised of two main components, enamel and dentine which are discussed in the following sections:

### **2.3.2.1 Enamel**

The outer structure of each tooth is made of enamel, which is the hardest tissue in the human body. The major component of enamel is mineral, constituting 96% of the tissue by weight, and the remainder is comprised of organic materials and water. The main mineral composition of enamel is calcium and phosphorus hydroxyapatite, which form a tight group of crystals. Due to the nature of these highly complex crystals, enamel is harder and stronger than other dental tissues. [244, 253] It is known that mineralisation increases from the EDJ to the surface enamel. [253] Enamel is the outer layer of the tooth crown. Two main structures are involved in enamel development. The first are enamel rods or prisms, which are groups of crystals formed by the ameloblasts during calcification in dental development. Across prism widths, a light and dark banding can be observed in the longitudinal sections of enamel, which is referred to as cross striations. Prism cross striations are structures of enamel and have been known to be related to the formation and growth of enamel. These striations are likely to be related to daily increments in matrix production, and the interval between lines is about 4µm. Sections observed in some studies using Scanning Electron Microscope (SEM) exhibited bright and dark bands across prisms. Enamel sections were treated and diluted with acid accentuates, and SEM showed the compositional contrasts of cross striations believed to be caused by variation in the mineralisation process, specially the amount of phosphate and carbonate in hydroxylapatite. [239, 244, 254, 255] Many histological studies have accepted that these cross striations along enamel prisms can be used as daily incremental markings in both permanent and deciduous teeth. [255-258] In primates, it is known that the matrix between bright and dark bands of cross striations correspond to about 24 hours of formed enamel matrix, a period known as a circadian growth rhythm. [259-261] Counting cross striations in individual teeth from formation to completion of enamel corresponds well with the estimated timing of tooth crown formation. [262] Moreover, Mimura (1939), Bromage (1991) and Dean (1998) used teeth from experimental animals that had been injected with markers such as sodium fluoride and lead acetate [263-265]; Smith et al

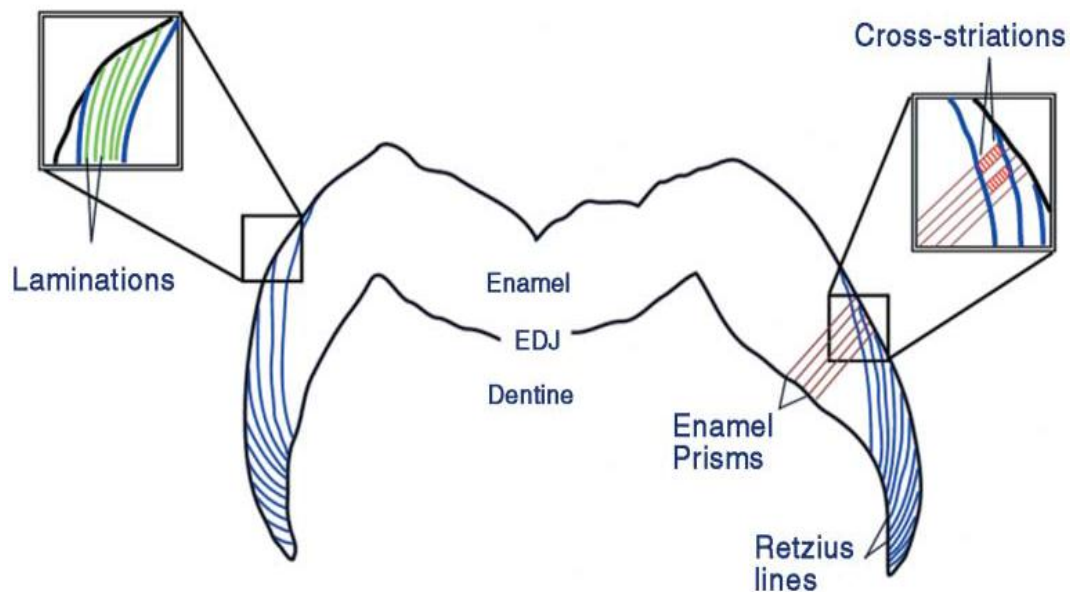
(2006) used known age primates [266]; and Antoine (2009) used modern human teeth from the permanent dentition [267], and these studies also indicated that cross striations reflect a circadian rhythm in enamel matrix secretion.

The second periodic structures in enamel are the brown Striae of Retzius, (named after a Swedish anatomist: A.Retzius, 1804-1860), which are prominent incremental lines resulting from variation in enamel mineralisation. Microscopic features of these lines show a series of dark bands running obliquely from the EDJ upwards and outwards to the surface. These lines form from changes in diameter of Tomes' processes, and the lines demonstrate the growth of enamel, similar to the annual rings on a tree on transverse sections of enamel.[254, 268]

The mineralisation of enamel and dentine follows a regular incremental pattern. The physiological activity of ameloblasts results in the formation of the neonatal line, an accentuated line forming within the pattern of striae. [239, 243, 256, 269-271] The neonatal line is formed at birth, and can be seen in a polished thin section of a tooth. This neonatal line can be used as a marker of the stage of tooth development at birth. After birth, the neonatal line clearly separates enamel formed before and after birth. Schour and Poncher (1937) investigated the rate of growth of enamel and dentine in human teeth by means of injections of doses of sodium fluoride. Each injection of sodium fluoride provided an obviously accentuated line in both enamel and dentine, starting at the time of injection. This experiment proves these distinctive lines in deciduous teeth are formed during the perinatal period. [272] Silness (1969) examined variations of enamel mineralisation in deciduous teeth using microradiographic and light microscopic methods and concluded that prenatal and postnatal enamel close to neonatal line mineralised to the same degree. The neonatal lines or birth lines in primary tooth enamel can be used to compare the enamel mineralisation before and after birth. [273]

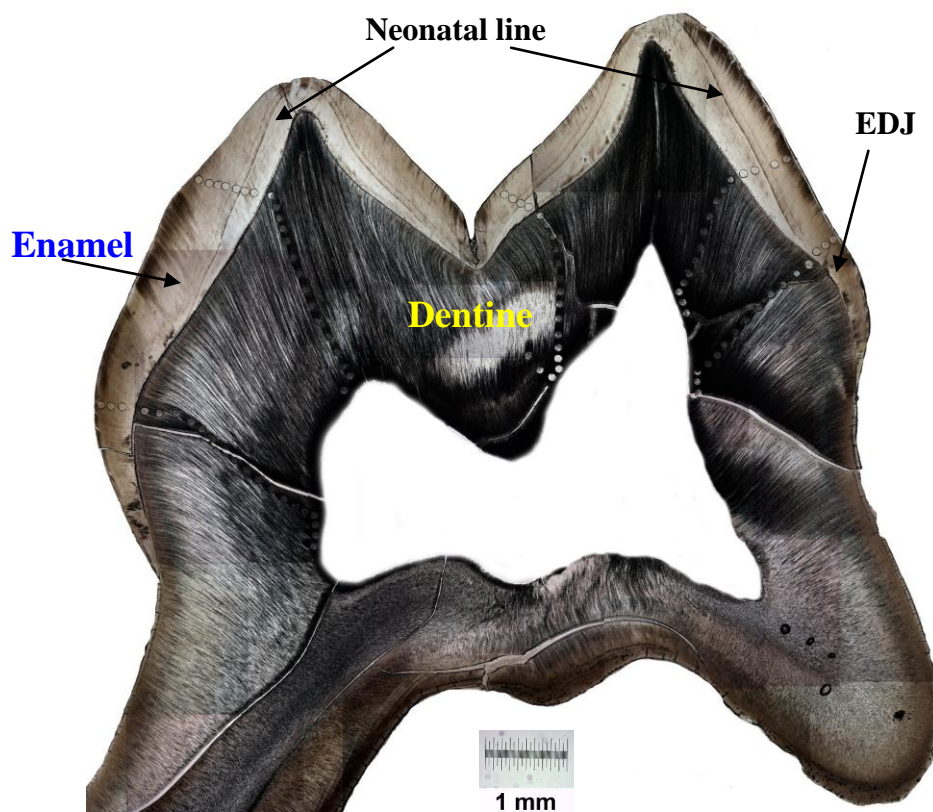
Enamel provides a record of metabolic changes during its development from the gestation period up to a year after birth.[274] Primary teeth start to mineralise between 13 and 16 weeks of gestation and continue to develop until the first year of life. [271, 275] Schour (1936), in a histological study, found 90% of all primary teeth have an obvious line corresponding to the time at birth. This distinctive line in enamel corresponds to a line in dentine. The neonatal line is found in all primary teeth and in the first permanent molars

due to this tooth initiating at the end of foetal life. [271, 275] Figure 2.6 shows incremental enamel features.

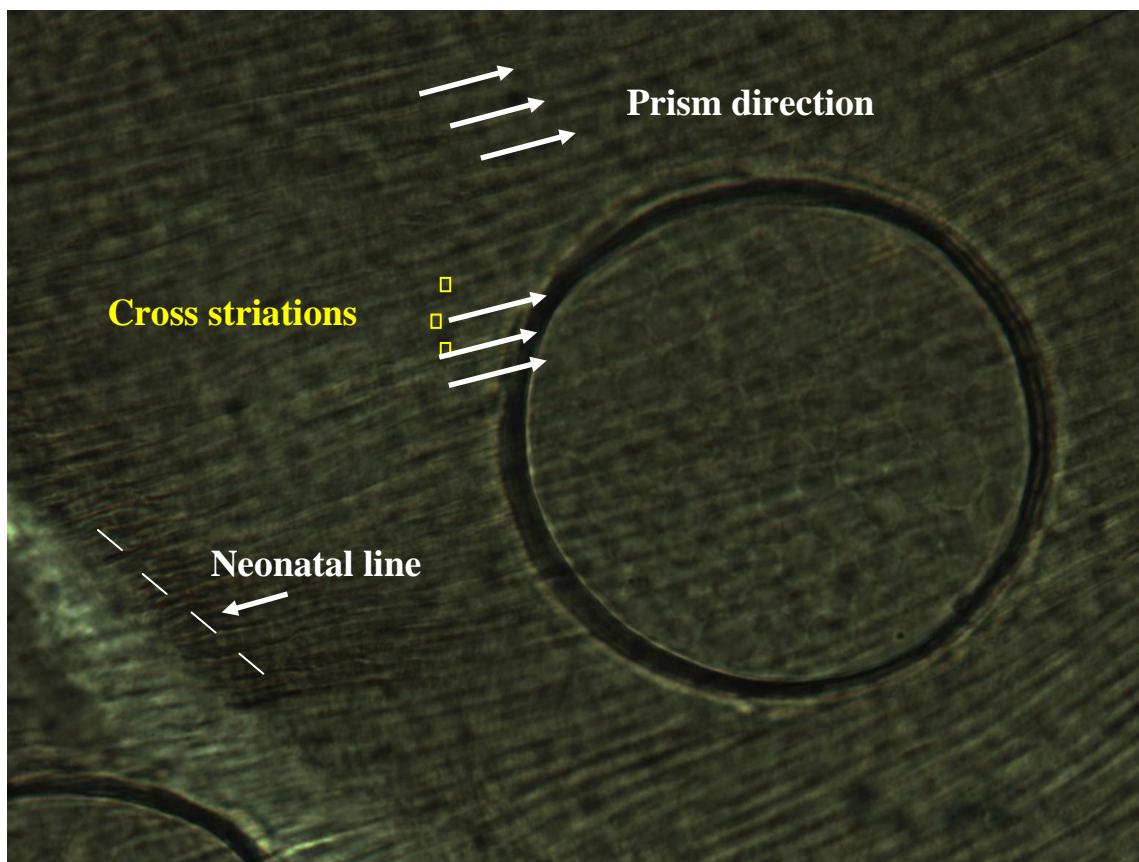


**Figure 2.6** Incremental enamel features From Smith, T. (2006) ‘Experimental determination of the periodicity of incremental features in enamel’. *J.Anat* Vol. 208, pp.99–113. [256]

The neonatal line in tooth enamel appears obliquely across the enamel prisms to the surface enamel. The neonatal line is usually much larger and darker than the growth lines. This is due to various physiological changes at birth.[239, 243, 250] Furthermore, the distribution of elements on each side of neonatal line can differ due to changes in the pre- and post-natal environments. [13] In the present study, the neonatal lines were used to identify birth and determine the age of the child at later stages of growth of the tooth using the subsequently forming normal growth increments as shown clearly from tooth sample taken from this study (Figure 2.7 and 2.8). More information is given in the section on age-related characteristics of dental histology.



**Figure 2.7** Neonatal line in longitudinal section of a deciduous tooth



**Figure 2.8** The cross striations in enamel in the present study

### 2.3.2.2 Dentine

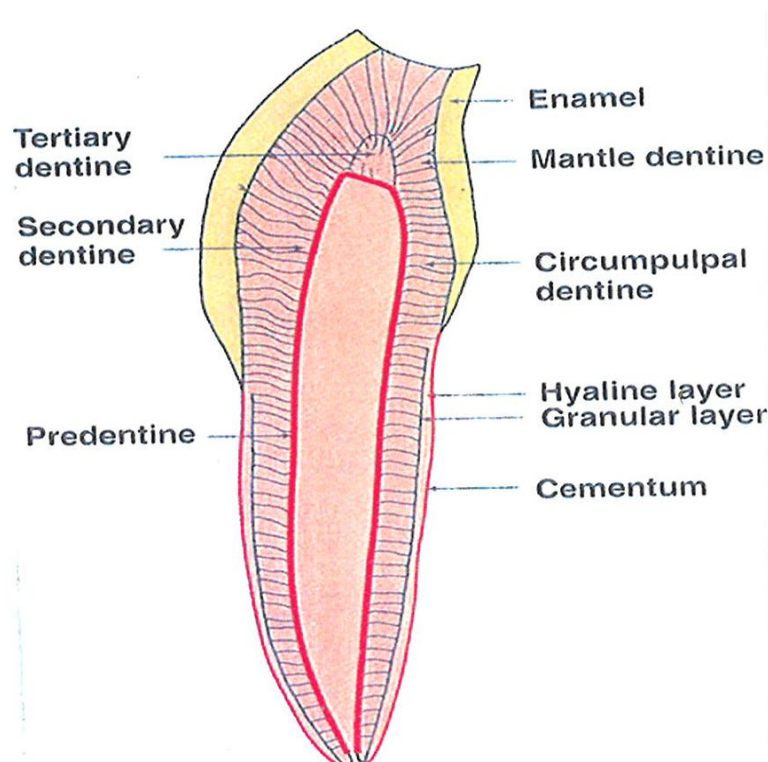
Dentine is a mineralized tissue formed throughout the life of a tooth. It is covered by enamel and cementum at the crown and root of the tooth respectively. The composition by weight of most dentine is 70% inorganic, 20% organic and 10% water. Most of the organic constituents are type I collagen and protein. Dentine is less hard, and more elastic and permeable than enamel. [239] Dentine formation is known as dentinogenesis, and is initiated just before enamel formation initiates. Dentine is classified by either the time of its formation or the region of the tooth in which it is found. Dentine types based on the time of development are the predentine, primary, secondary and tertiary dentine. Dentine types based on the region of the tooth include coronal dentine and root dentine. Coronal dentine includes mantle and circumpulpal dentine, and the latter comprises globular, intertubular, peritubular or intratubular, and sclerotic dentine. Odontoblasts are dentine-forming cells, which differentiate from the cells of the dental papilla. These cells start secreting organic matrix (usually collagen fibres) to establish the first dentine layer, called the 'mantle dentine'. After the mantle dentine has been mineralised the dentine gradually develops to become a thick layer. Odontoblasts continue to differentiate and lay down 'circumpulpal dentine matrix' which consists of collagen fibrils. The mantle and circumpulpal dentine are major parts of the primary dentine. When the tooth root has developed fully, odontoblasts form the secondary dentine, usually at a very slow rate depending on tooth type and age of development. In general, it seems that there is little difference between the morphology of primary and secondary dentine, although secondary dentine may contain fewer tubules and can be identified by a change in tubule direction close to the pulp. In the area between the dentine and the odontoblasts which line the pulp, there is a layer of predentine. It is this innermost layer where new dentine is laid down throughout life. Although dentine development occurs throughout life, tertiary dentine is formed only in reaction to stimuli such as cavity preparation, caries and tooth attrition. [239, 250, 276] Figure 2.9 illustrates the different regions of dentine.

Primary dentine has a tubular structure formed during dentinogenesis as the odontoblast processes secrete matrix around themselves. These tubules persist through the entire thickness of primary dentine from EDJ to the pulp area, and carry dentinal fluid and nutrients throughout dentine. In the crown, the tubules follow an S-shaped curve from the outer surface of the dentine to the pulp. A study reported measurement of the diameter and number of the dentinal tubules in animal and human dentitions using scanning



electron microscope. [277] In mineralised dentine there are thousands of microscopic tubules that extend from the pulp-dentine border to the EDJ or cement dentine junction (CDJ). Dentine tubules vary in diameter and volume depending on the age of the tooth and position within the dentine. The diameters of tubules in young teeth are between 1 and 3 $\mu\text{m}$ . The cross-section of 1mm<sup>2</sup> dentine contains about 40,000 dentinal tubules; there are about 20,000 and 60,000 tubules near enamel and the pulp chamber respectively. There are more tubules per unit area near the pulp, and these tubules are wider (2-3 $\mu\text{m}$ ) than near the enamel (1  $\mu\text{m}$ ). The tubules are more separated near enamel and more tightly packed near pulp so the permeability of dentine increases nearer the pulp.[239, 243, 244, 277]

Under the gingiva, the dentine of the root is covered with a thin layer of calcified tissue called cementum. This is softer than enamel and dentine due to being comprised of collagen fibers and less than 50% hydroxylapatite. The function of cementum is to bond the periodontal ligament to the surface of the root. [239, 244]



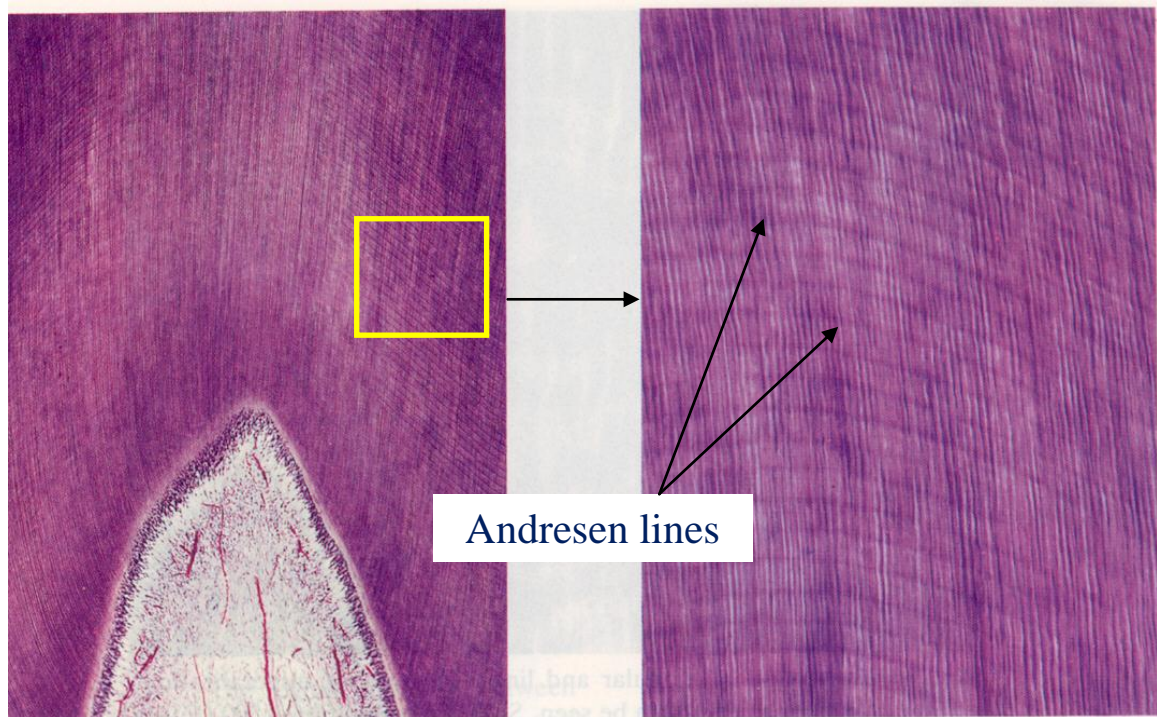
**Figure 2.9** The regions of dentine

From Berkovitz, BKB., Holland, GR., Moxham, BJ. (2009) *Oral anatomy, histology and embryology*. 4<sup>th</sup> ed, Edinburgh: Mosby. [239]

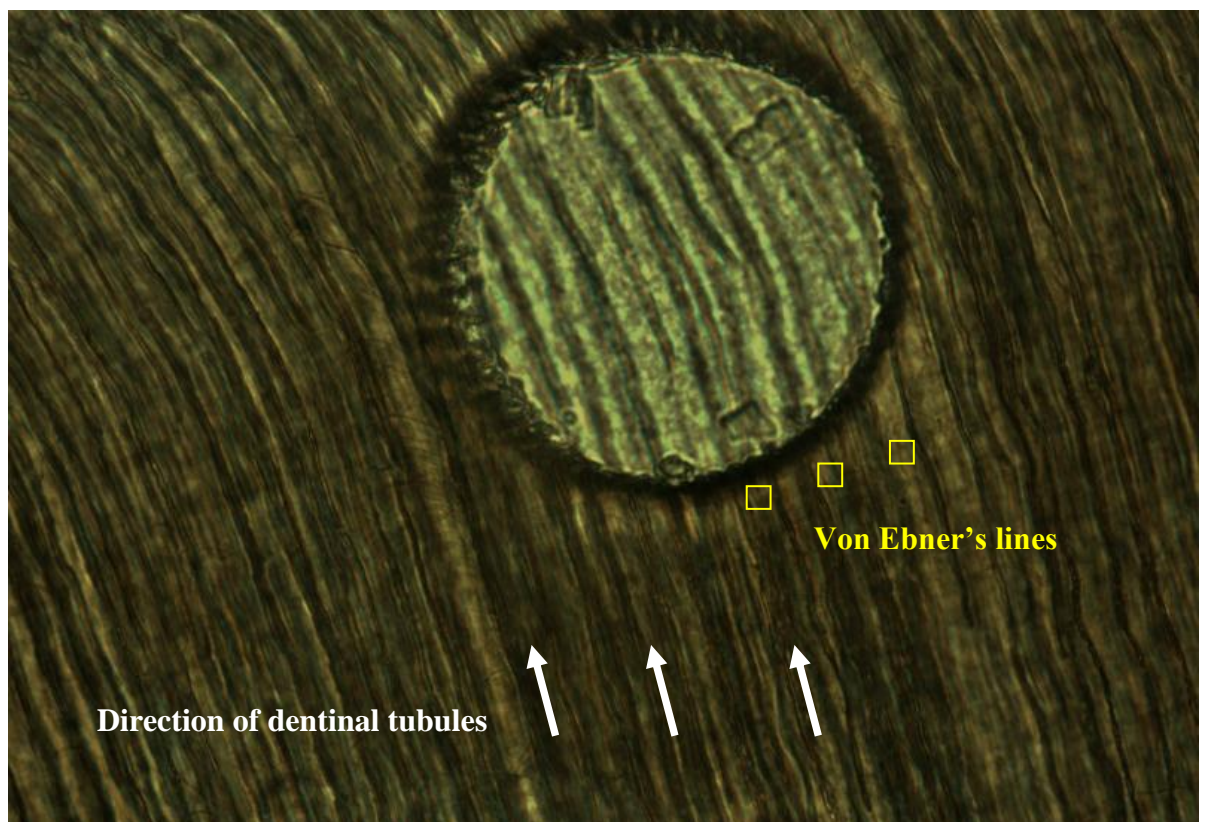


Enamel, dentine and cementum differ in the size of their hydroxylapatite crystals and in their overall proportion of organic to inorganic constituents. Collagen is frequently found as the principal organic component of dentine and cementum, but there is no collagen in enamel. Proteins secreted during enamel formation are almost wholly displaced during the process of enamel mineralisation. [239, 244]

Dentine formation is complex. It involves predentine secretion followed by mineralisation of predentine. Secondary dentine is then formed adjacent to the pulp. These processes occur at different rates and times. Previous studies argued that incremental lines in dentine may reflect physiological rhythms related to mineralisation, but these markings are difficult to interpret. [269, 278, 279] Two sets of incremental lines in dentine are visible using polarized microscopy or electron microscopy are short and long period lines. Short-period lines in dentine, or daily markings, refer to von Ebner's lines. Long-period lines are known as Andresen lines in dentine. [280] Dentine is formed by odontoblasts that generate dentine tubules (equivalent to enamel prisms), and show daily incremental lines known as von Ebner's lines (equivalent to cross-striations in enamel) and long-period structures known as Andresen lines (equivalent to Retzius lines in enamel). Previous studies demonstrated that both cross-striations and von Ebner's lines show a 24-hour frequency, and because they are easier to image than von Ebner's lines, cross-striations are used as a standard to determine the periodicity of short-period features in enamel and dentine. Long-period lines seem likely to show a consistent periodicity within a single tooth and in all teeth belonging to the same individual. Counts and measurements of these short- and long-period lines in both tissues provide informative data on the rate and duration of enamel and dentine secretion, which may be able to be used to determine the total crown formation time and the rate and duration of root extension. [269, 278, 280] Figure 2.10 shows the long period lines in dentine and Figure 2.11 shows short period lines in dentine in the present study.



**Figure 2.10** Long period lines or Andresen lines in dentine  
 [Picture courtesy of Doug Luke]



**Figure 2.11** Short period lines or von Ebner's lines in dentine in the present study

### 2.3.3 Age-related characteristics of dental histology

Teeth are formed chronologically, and can be used to estimate age in both animals and humans. [240, 244, 257] Evidence of development rates in the tooth can be gathered from the incremental lines that exist in both enamel and dentine. Measurement of these lines reveals the development of dental hard tissues from initiation to the completion of the root. [239, 244]

Similarly to other biological tissues, the development of hard tissues follows a circadian period that duration of which can be identified by incremental growth lines. These growth lines steadily record the rates of tooth development in enamel and dentine. [239] Previous studies have indicated that the distance between von Ebner's lines corresponds to the daily rate of dentine formation, and so this measure is therefore used to infer rate of development and replacement in the tooth. [269] These lines are a well preserved indicator of the time of human development, and the investigation of these lines can be used to reconstruct the timing of tooth formation. [245, 260, 269, 281]

In deciduous enamel, daily incremental lines range between 2.5 and 4.5 $\mu$ m, but showed a marked decrease in the area immediately following the neonatal line. [255] Daily incremental lines in coronal dentine of permanent teeth are deposited at 1-2 $\mu$ m daily near the enamel dentine junction, then increase to 4-5 $\mu$ m per day, and then fall again near the pulp cavity. [260] Dean and Scandrett (1996) studied incremental markings in the dentine of human permanent teeth and found that long-period incremental lines had a consistent periodicity in an individual, and can be used with confidence to construct the timing of tooth development in many fields including forensic, archeological and paleontological studies. [269]

Information about incremental growth lines in enamel has been used to reconstruct tooth development, and the method was first used in calculating the age at death of an archaeological specimen. [244, 282, 283] More recently incremental lines in enamel have been used to explore the history of nutrition, weaning stress, heavy metal exposure and hormone activity in humans and animals. [13, 240, 245, 246, 255, 284]

The neonatal line has been used to study the distribution of lead levels across deciduous tooth surfaces, showing the intensities of lead exposure during the pre- and postnatal

periods of development [13, 240] The neonatal line is clearly visible in enamel and can be used to identify different periods of enamel formation and to construct the chronology of tooth development. Two key studies are described below which used incremental growth lines in enamel in a combination of LA-ICP-MS in order to construct the history of trace element incorporation into enamel in early life.

Humphrey et al (2008) first demonstrated that incremental lines in the enamel of deciduous teeth were likely to be useful for studying the pattern and timing of calcium-normalised strontium (Sr/Ca) ratios in children to reconstruct the history of weaning. The technique measured the distance along the enamel prism, and the age of each ablation point was derived by counting daily lines along the prism, continuing the counts out along new prism paths to each ablation point in turn in the chronological sequence. Finally, the number of days were derived and determined as intervals in order to show the change of Sr/Ca ratios over time. [245] In another major study, Dirks et al (2010) applied this technique in order to study weaning stress in baboons, and found that the ages at which significant stresses during the weaning process occurred could be identified using accentuated lines in enamel and matched to changes in Sr/Ca. [246]

Although enamel has characteristics that can be used in constructing a chronological record of early life, enamel records the period of crown formation, which varies from tooth to tooth and is shorter than the time recorded in dentine, which continues to form after the enamel of the crown is complete. [285] Dentine mineralises slowly throughout early life, and its chronology covers the whole process from crown development to root completion. The periods of dentine development are therefore longer than those for enamel, implying that the history of trace element incorporation could be better assessed using dentine. [239, 244] Moreover, previous studies have found that significantly higher trace elements such as lead were found in dentine compared to enamel [13, 241, 286], and therefore in the present study, we believed dentine had potential for reconstructing the history of lead exposure.

### **2.3.4 Lead incorporation in enamel and dentine**

It is well known that lead accumulates in calcified tissues, including teeth. [13, 252] A study in rats undertaken by Kato (1977) reported that intravenous injections of lead produced a 'lead-line' in the bone and dentine of rats within 30s. There was also an increase in serum calcium and phosphorus concentrations, with maximum observed values at 1 hour and 30 minutes. The findings of this study suggest that the replacement of calcium with lead in the hydroxyapatite could have changed the configuration of crystal lattices, resulting in phosphorus and calcium being released into the blood to restore the stability of the calcium apatite. [287]

It has been known that enamel is a resistance tissue upon maturation process. Mature enamel does not remodel and can be used to record the incorporation of trace elements into its matrix during enamel formation of early life. [288, 289] Primary teeth provide chronological record the prenatal and early postnatal periods. [240, 245] Thus, researchers are able to use dental tissues including enamel to explore the distribution of trace elements, nutritional status and diseases during the prenatal and postnatal periods.

#### **2.3.4.1 Enamel mineralisation**

Starting at the cusp tips of the crown, dentine forming cells known as odontoblasts move from the EDJ toward the future pulp cavity leaving behind a protein matrix referred to as predentine, which will later calcify to form fully developed dentine. Concurrently, ameloblasts move in the opposite direction, away from the EDJ and forward to the tooth surface, also leaving a matrix protein. While the enamel is still matrix, there is already a mineral component. Mineral is secreted in long thin ribbons in a lattice of proteins that constrain it so that it grows in length but not width. During maturation, the crystals become thicker as the organic protein lattice is removed. When the full thickness of enamel is reached at any given part of the tooth crown, the enamel matrix withdraws any protein and water, resulting in the enamel hardening to 96-97% mineral composition by weight. The direction of full mineralization refers to the waves of calcification that move from the tooth surface to the EDJ and back to the tooth crown again, resulting in a greater mineralisation at the outer layer of enamel. Trace elements are incorporated in hydroxyapatite crystals during the period of enamel development, and enamel has been

used to identify the uptake of nutrients such as zinc, strontium, calcium [253, 289-291], and lead exposure. [13, 253, 288] Recent studies have used incremental lines in enamel to reconstruct the chronological record of tooth development, and identify diet history and stress [245, 246], as described in Section 2.3.3.

#### **2.3.4.2 Lead levels in enamel**

Previous studies on lead in different components of human teeth indicate that lead occurs at comparatively low levels in the deep enamel and increase sharply toward the enamel surface [253], with various studies indicating high surface enamel lead concentrations. [5] Unlike other dental tissues, enamel is not remodelled because it is acellular and its high levels of hydroxylapatite. [250] Enamel maturation is completed in the subsurface enamel before the tooth emerges into the mouth. Trace elements such as lead incorporated into the mineral component of the subsurface enamel therefore reflect environmental exposure before the age of emergence. High levels of trace elements in surface enamel are then hypothesized to occur due to ion exchange with the tissues surrounding the tooth prior to emergence and, after emergence, to ion exchange in the oral environment. For example, every time we drink a coca-cola or orange juice, the acid in the drink dissolves some of the hydroxyapatite. The saliva buffers the teeth because it has a higher pH so that calcium ions and other components of hydroxyapatite recrystallise the surface of the tooth. At this time, any lead that is coming through the mouth or is in the saliva from other sources of exposure is incorporated into the surface enamel. [197, 240, 253]

In addition to studies of lead levels in human teeth, there has been increased interest in animal studies. In a study of rats exploring the effects of exposure to lead on dental enamel formation, a delay in mineralised enamel development was found. [292] A recent study of lead in the tissues of lead-dosed goat teeth suggested that circumpulpal dentine was the most appropriate tissue from which to sample in assessing cumulative lead exposure. [293] However, there is as yet no consensus on the most meaningful measure of lead from different parts of the human tooth.

### 2.3.4.3 Dentine mineralisation

Dentine is composed of an organic matrix of collagen fibres and mineral hydroxyapatite. Generally, there are two stages of dentine formation: organic matrix secretion and mineralisation. At the secretion stage, odontoblasts, the dentine forming cells, synthesize and secrete an organic matrix consisting especially of type I collagen and proteoglycans, and other crucial constituents of the predentine layer. The predentine is a layer of unmineralised matrix 10-30  $\mu\text{m}$  wide which is seen between the area of odontoblasts and the mineralized dentine. In predentine, collagen molecule fibres aggregate with their long axes in parallel to become fibrils, which further rearrange into bundles. [239, 244, 250, 290]

Coronal primary dentine matrix is synthesized at a rapid rate during tooth development. Thus, the bulk of the crown consists principally of primary dentine, which outlines the pulp chamber and is therefore referred to as circumpulpal dentine. At the outermost layer of the primary dentine, under the enamel, a narrow zone called mantle dentine occurs. This is a product of the first mineralisation reaction by newly differentiated odontoblasts, and it has a composition slightly different from circumpulpal dentine. Mantle dentine is composed of large collagen fibres approximately 0.1-0.2 $\mu\text{m}$  in diameter. [290, 291] Mantle dentine is less mineralised, and contains no growth lines. The main bulk of the dentine which underlies the mantle dentine is termed circumpulpal dentine, which is approximately 6 to 8mm thick in the crown and a little thinner in the roots. During tooth formation, circumpulpal dentine arises immediately after mantle dentine formation. [239, 244, 250, 290]

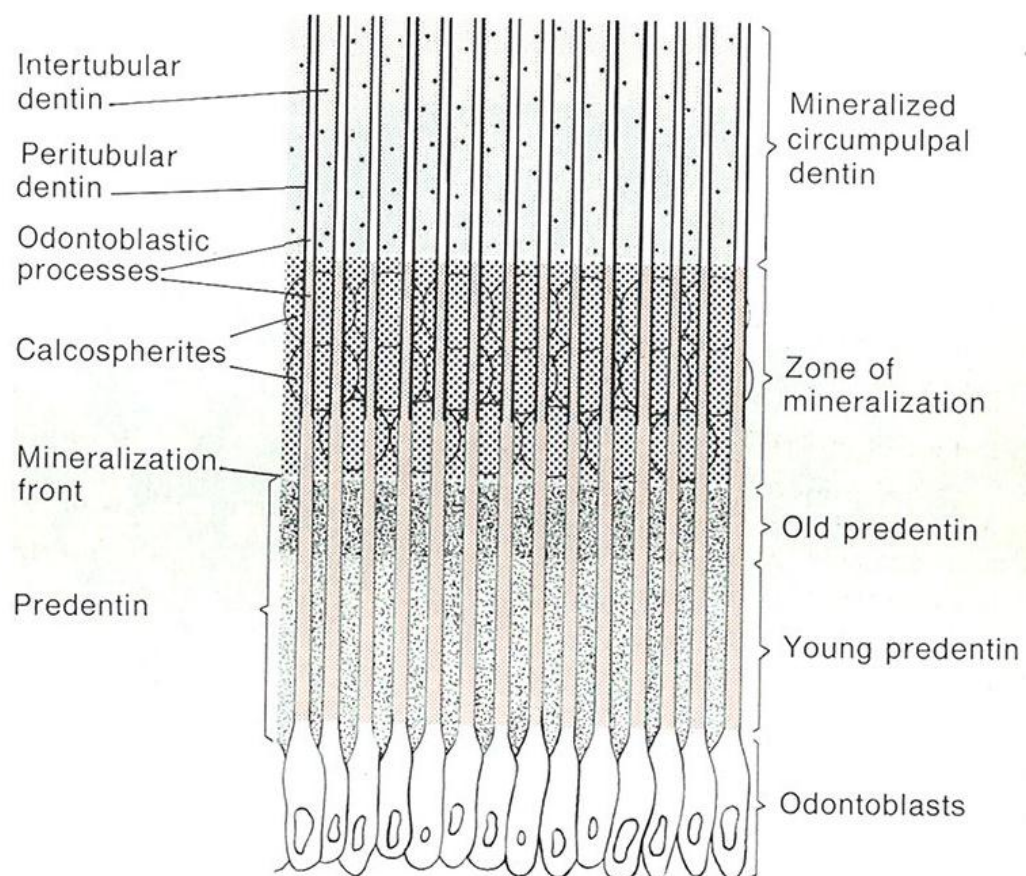
After matrix secretion, a second simultaneous process of formation of an inorganic phase continues at the mineralization front. Calcium ions are transported to the mineralisation front by a transcellular route, which includes ions, minerals and organic materials to maintain calcium ions in the odontoblasts. At the mineralization front, the mineralization of the entire circumpulpal dentine occurs, involving the growth of 'calcospherites' or 'calcium globules'. These globules are the principal structures of active mineralization observed under a scanning microscope, and the ions and minerals increase apatite formation. They bind to the collagen fibre surface and enhance the ability of the fibres to bind calcium ions, resulting in higher mineral content. It has been found that faster rates



of secretion of predentine increase calcospherite formation, especially at the mineralising front, leading to interglobular dentine formation. [239, 244, 250, 290]

Following primary dentinogenesis and after root formation is complete odontoblasts continue to deposit secondary dentine around the pulp at a slow rate, leading to the reduced size of the pulp chamber. Structurally, secondary dentine resembles primary dentine, also having a tubular pattern which is, however, less regular than that in primary dentine. As a reaction to various external insults, such as dental caries, attrition and trauma, tertiary dentine or reparative dentine is also synthesized. [239, 244, 250, 290]

Figure 2.12 shows the pattern of dentine mineralization.



**Figure 2.12** The pattern of dentine mineralization

From Mjor IA, Fejerskov O. (1986) *Human Oral Embryology and Histology*, Copenhagen: Laursen-Tonde. [290]

Evidence indicates that when teeth are mineralized, the inorganic content of enamel and dentine is likely to change along with changes in the blood and saliva. Due to its lower pH, lactic acid results in more demineralisation than remineralisation, and minerals are



lost resulting in caries. [294] In such circumstances, the enamel surface can release calcium, phosphorus and fluoride ions into the saliva at lower pH and, in a similar manner, other minerals including lead may also be lost from the enamel surface. Research undertaken by Gulson and Gillings (1997) using stable lead isotopes in 47 European migrants in Australia found that the dentine adjacent to the pulp can exchange lead at a rate of about 1% per year. However, no significant lead exchange was observed in the other part of the dentine. [295]

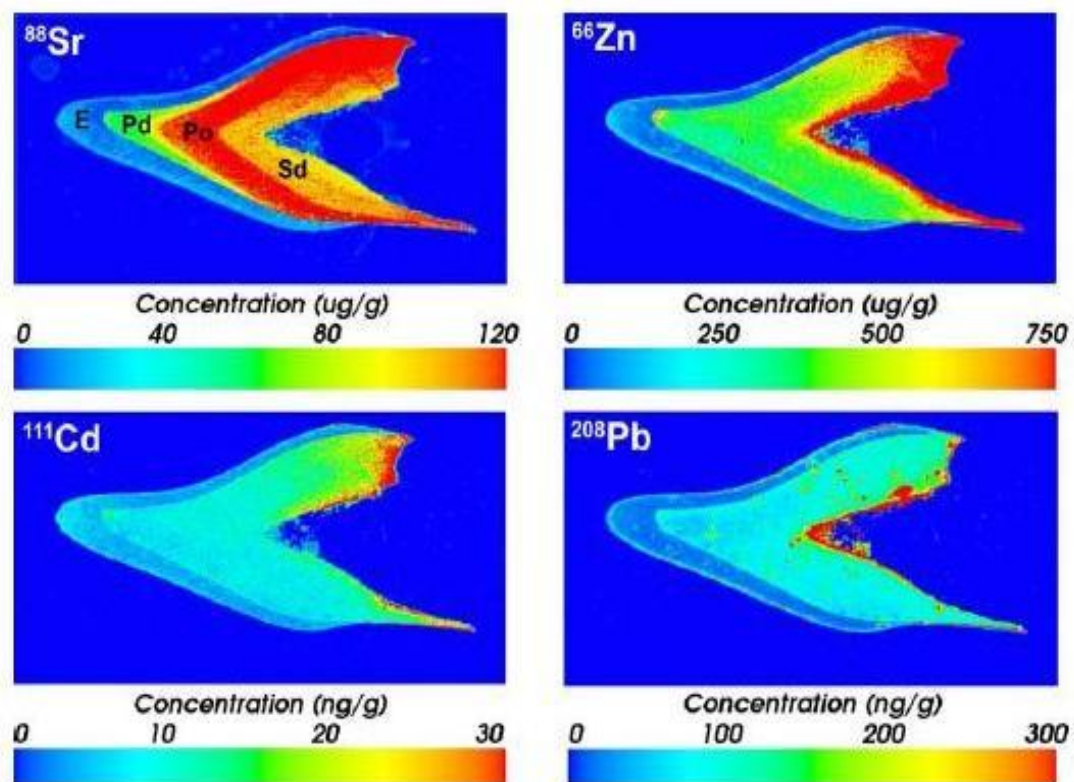
#### **2.3.4.4 Lead levels in dentine**

Because dentine mineralises throughout the life of the tooth, lead sampled from this tissue may represent long term or cumulative exposure. [13, 14, 286] Globler et al (2000) and Arora et al (2006) found that lead in primary dentine is present at higher levels than in enamel. [13, 241] Arora examined prenatal and postnatal dentine close to the EDJ and cemento-enamel junction (CEJ) respectively, and the results showed differences in lead accumulation in pre- and postnatal development. A study of lead isotopes in teeth in Australian immigrants reported that the rate of lead exchange from reservoirs in circumpulpal dentine was low, at about 1% per year. [295]

Recent studies of lead levels in different parts of the teeth include Kang and Arora. Kang et al (2004) used LA-ICP-MS to determine the micro spatial distribution of metals, including lead, in the exfoliated deciduous upper central incisor from a child living in Mexico. The areas in the tooth which were ablated for lead concentrations were prenatal enamel, the neonatal line, postnatal enamel, the enamel-dentine junction, dentine, and the dentine-pulp junction. Elevated lead levels were found in the neonatal line and dentine-pulp areas. [286] Arora et al (2006) measured lead intensities in the pre- and postnatal enamel and dentine of the primary teeth of 10 schoolchildren aged between 6-12 years in Australia. Children were categorised into two groups: high ( $>10\mu\text{g/dL}$ ) and low blood lead levels ( $<10\mu\text{g/dL}$ ). Mean blood, dentine and enamel lead levels were measured in both groups, and lead levels in both blood and postnatal dentine statistically differed between the groups while there was no significant difference in mean enamel lead levels. From these studies, it seems likely that lead levels in dentine are a more sensitive marker of exposure than those in enamel. [13]

Recently, Geographic Information System (GIS) and Interactive Spectral Imaging Data Analysis (ISIDAS) have been used to map the distribution of elements on the tooth surface. GIS was first used to map levels of calcium normalised strontium in enamel of primary teeth to reveal the dietary history of children. [245, 284]

Studies using these techniques have demonstrated an increasing concentration of lead in dentine on approaching the pulp cavity, according to Kang (2004), Arora (2006) and Hare (2011). [13, 286, 296] A study of element bio-imaging in deciduous incisors using LA-ICP-MS showed that concentrations of lead, zinc and strontium were higher in dentine particularly in areas near the pulp cavity as shown in Figure 2.13 [296] Furthermore, high lead levels adjacent to the pulp are also consistent with the distribution of manganese, as reported by Arora et al (2011). [297]



**Figure 2.13** Element distributions in deciduous incisors

From Hare, D., Austin, C., Doble, P., Arora, M. (2011) Elemental bio-imaging of trace elements in teeth using laser ablation-inductively coupled plasma-mass spectrometry. *Journal of Dentistry*, 2011. 39(5): pp.397-403. [296]

## 2.4 Measurement of lead in teeth and blood

The advantages and limitations of using dental lead levels for lead exposure in epidemiology studies are discussed in the following sections.

### 2.4.1 Lead levels in teeth

Previous studies have reported the use of lead in teeth as a biomarker of past exposure among children. Over the past three decades, these studies have most often analyzed whole teeth, which have been digested in acids. [156, 237] A comparison of lead levels in whole primary teeth from different countries is shown in Table 2.3. The differences in lead levels by country are likely to be due to various reasons, such as different types and levels of environmental exposure over different time periods, the heterogeneity of sample sources, incompatible sampling processes [298] and different detection limits of measurement techniques. [237]

**Table 2.3** Comparison of lead levels in whole primary teeth from different countries

<b>Author, Publication year [Ref]</b>	<b>Place</b>	<b>n</b>	<b>Methods</b>	<b>Lead levels (mean <math>\pm</math> SD) (<math>\mu\text{g/g}</math>)</b>
Arruda-Neto, 2009 [299]	Brazil	50	ICP-MS	$1.28 \pm 0.11$
Aromary, 2006 [118]	Jordan	268	AAS	$28.91 \pm 13.70$
Hernandez-Guerrer, 2004 [300]	Mexico	100	AAS	$8.28 \pm 6.18$
Rahman, 2002 [301]	Pakistan	138	AAS	$16.8 \pm 6.29$
Bayo, 2001[302]	Spain	371	Microwave oven acid digestion	$3.5 \pm 1.7$

Although tooth digestion provides information about cumulative lead exposure, this approach tells us nothing about the timing or history of exposure. Because it is known that different components of human teeth mineralise lead differentially, analyses of whole teeth do not allow the time specific distribution of this metal to be assessed. [286] There is therefore increased interest in studying lead exposure in different parts of the tooth to enable this mineralisation over time to be assessed.[13, 286]

Different parts of the tooth have been studied for lead exposure, and differential levels of lead have been identified. [13, 240] Gulson (1996) determined that lead exposure in

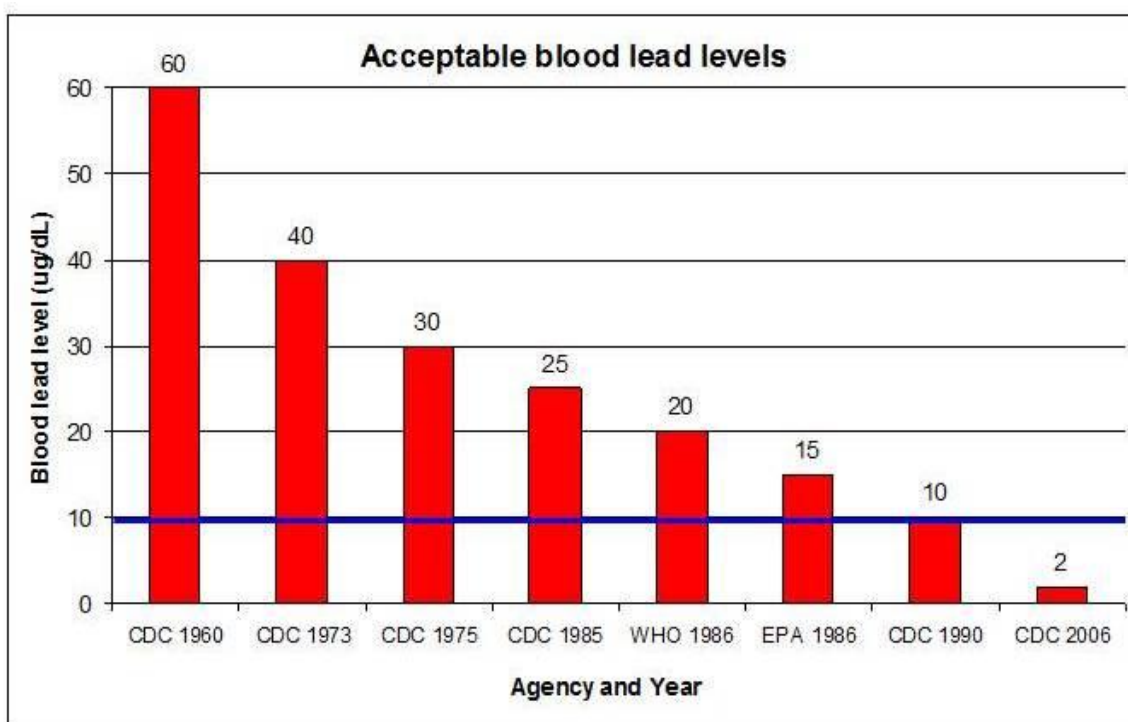
human deciduous teeth enamel best reflects in utero exposure, while dentine gives a measure of exposure in early childhood. [242] Another study of lead levels in primary teeth aiming to develop an appropriate biomarker for prenatal and neonatal exposure from birth to 1 month found that the distribution of lead levels in the dentine provides a marker for exposure during both pre- and neonatal development. [13]

## **2.4.2 Tooth-lead levels and health outcome**

Tooth lead levels have been used to study the association between chronic low-lead exposure and health effects. Longitudinal cohort studies evaluating tooth-lead levels and health have mainly focused on the effects of lead on neurological development, IQ and behaviour. [10, 303] Needleman and colleagues found that children with high dentine-lead levels measured by tissue digestion rated poorly on teacher-reported measures of classroom behaviour. [76] A subsequent study also found that high dentine lead concentrations were associated with decreased IQ scores. [78] Several cross-sectional studies have also studied the association between tooth lead levels and neurological development, some reporting similar effects on neurological development, [127, 304] other finding no effect after controlling for potentially confounding social variables. [80] Some studies have determined associations between tooth-lead levels and other health outcomes including chronic renal failure [305] and dental caries, especially in primary teeth, [113, 306, 307] as discussed in Section 2.1.6

## **2.4.3 Blood lead levels**

Blood lead level is the most commonly used biomarker of lead exposure. The effects of lead are typically described in relation to blood lead levels, and therefore the relevant standards are also defined in terms of blood lead. The standard for both children and adults was 60 µg/dL until the 1960's, which progressively reduced to 25 µg/dL in 1980s. The current threshold level for health is a BLLs of 10 µg/dL. After phasing out of leaded petrol, BLLs surveyed in various developed countries were found to have decreased. Figure 2.14 shows the acceptable childhood BLLs proposed by several organisations over time.



**Figure 2.14** Acceptable BLLs proposed by agencies at different times

## 2.4.4 Correlation between tooth and blood lead levels

Various studies have indicated a positive correlation between tooth and blood lead levels. For example, a study of 18 preadolescent African-American children showed a high positive correlation between blood and whole deciduous tooth lead levels with  $r = 0.836$  ( $p < 0.01$ ). [308] A study of 23 school children living in Belgium also showed significant correlations between lead levels in permanent tooth surface enamel and blood, particularly for the pre-eruptive incisor component ( $r=0.50$ ,  $p<0.05$ ). [199] On the other hand, a study of 60 Egyptian school children by Omar (2001) reported that the correlation between whole primary teeth and blood lead levels was not statistically significant. [156]

When lead is elevated in blood, it is distributed around the body and ultimately retained in tissues such as teeth and bone. [241] Lead levels in teeth and bone have a long half life, whereas BLL reflects only recent exposure. Because of this, it is still difficult to compare tooth and blood lead levels in humans. Moreover, results from different studies are difficult to compare because sampling techniques and analytical methods used to assess the tooth-blood relationship are different in different studies. [241] For

instance, some blood samples were collected by finger prick or venipuncture, [20] and some studies analysed whole teeth whereas others selected specific parts of the tooth such as enamel or dentine.

As noted above, there are variations in lead levels between persons, countries and specific human tissues. Over the last decade there have been attempts to assess lead levels in different matrices including blood and teeth, especially in children. For example, a cross-sectional study of 60 children aged 6–12 years in urban and rural areas in Egypt, mean BLL in urban areas were higher than rural areas. Socio-economic status was not associated with tooth and blood lead levels. A history of wrapping sandwiches with newspapers and the age of the child were significant indicators of lead exposure in children living in urban areas, the distance between home and school was statistically significant among children in the rural areas. The authors suggested that teeth lead levels can be used to assess the body burden of environmental lead. [156] Another study measured lead concentrations in teeth and blood in South African children. Dentine lead levels were significantly higher than those found in enamel, and circumpulpal dentine had the highest lead levels. But an association between tooth and blood lead concentration was not compared due to lack of previous data. [241] In addition, lead levels in different part of the tooth should be considered in order to assess an association between tooth and blood lead levels. Some previous studies have found different levels of lead in each tooth structure [13, 156, 241, 286, 288], with some finding higher lead levels in dentine [13, 241, 286] and others finding higher lead levels in enamel. [288]

## **2.5 Principles of the methods used in this study**

### **2.5.1 Introduction**

Several methods are available for assessing the concentrations of lead in biological samples. These include Atomic Absorption Spectrophotometry (AAS), Electrothermal Atomisation AAS, Graphite Furnace AAS, X-ray Micro-Analyses, and Inductively Coupled Plasma Mass Spectrometry. One factor that is very important in selecting the most appropriate analytical method is quality assurance. Both accuracy and reliability of procedures are important. [6]

Atmospheric-pressure inductively coupled plasma (ICP) is an analytical technique used for the detection of trace metals in geological, environmental and biological samples. Generally, ICP combines three applications: Atomic Emission Spectrometry (AES), Atomic Fluorescence Spectrometry (AFS) and Mass Spectrometry (MS). These three main ICP-based techniques are applicable to nearly all trace elements. Among those techniques, it has been suggested that ICP-MS and ICP-AES are the most powerful methods for the rapid determination of multi-elements. [309]

This study involves biological samples of blood and teeth. Two techniques, ICP-MS and Laser Ablation Inductively ICP-MS, were used to measure the levels of lead in blood and teeth respectively. The following sections describe the basic principles of each method, relevant measurement issues, as well as the advantages and limitations of the methods.

### **2.5.2 Inductively Coupled Plasma Mass Spectrometry (ICP-MS)**

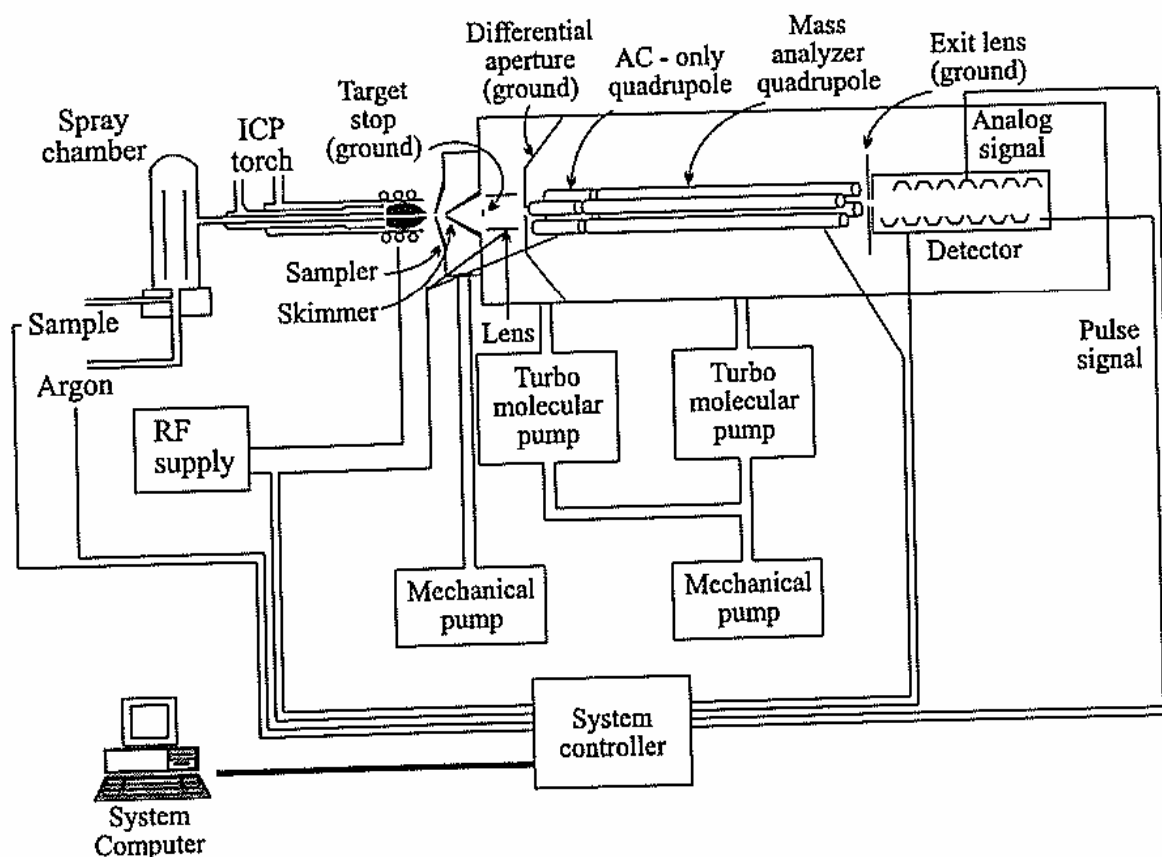
ICP-MS was first introduced by Houk et al (1980), who combined argon ICP with a quadrupole mass spectrometer for the multi-element analysis of aqueous solutions. [310, 311] After the release of commercial products in 1983-1984, this technique has been developed further and is now widely used for the elemental analysis of geological and environmental samples. [309, 311] ICP-MS is a highly sensitive technique for multi-

element and isotope analysis. In the present study, ICP-MS was used to measure blood lead levels in children.

### **2.5.2.1 Principles and components of ICP-MS**

ICP-MS can be used to analyse all elements. Detection limits have been found to be lower than 1 µg/L for 60 elements in a multi-element analysis. Moreover, this technique can be applied to all kinds of materials including solutions, solids, and gaseous samples. Solid samples are usually vaporised using laser ablation or heated cells. Solutions are generally vaporised using a nebuliser, whereas gases may be sampled directly. [309] The chemical analysis with inductively coupled plasma (a state of the matter including electrons and ionized atoms) is based on the crucial principles of vaporization, dissociation, and ionization of chemical elements when they are brought into the hot plasma. Then, elemental ions are produced in the plasma at varying temperatures between 5,000 and 10,000 K. [309, 311] More details for ICP-MS coupled with laser are described in the section 2.5.3. Figure 2.15 shows the basic components of ICP-MS.





**Figure 2.15** Diagram of basic components of ICP-MS

From Montaser, A. (1998) *Inductively coupled plasma mass spectrometry*. Wiley: New York. [309]

### 2.5.2.2 Advantages and Limitations of ICP-MS

The ICP-MS is a highly sensitive technique for examining multi-elements in different fields, including biological, geological and environmental samples, marine and atmospheric chemistry and volcanology. It is a fast, precise, accurate, and extremely sensitive analytical technique for determining of trace elements in liquid and solid sample materials. Lead concentrations can be detected in the range from ppb to ppm. The detection limits vary depending on size of ablation pit, which can be increased as required. This technique is a fast analysis due to multi-elements can be analysed at the same time. It also provides a simple operation method and cost of operation may be cheaper than other analytical techniques. [309, 312]

Although there are several advantages to using ICP-MS, disadvantages have also been reported. [233] ICP-MS is a destructive technique because an analysis of biological

samples requires liquefied samples dissolved in acid as such it is not an appropriate technique to use to assess lead levels in teeth if subsequent histological analysis is required, as in this study.

### **2.5.3 Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS)**

Alan Gray (1985) first used laser ablation for ICP-MS as a technique to measure solid samples using a finely focused laser. LA-ICP-MS has been used for the analysis of geological samples, providing information on their chemical and physical properties. Recently, LA-ICP-MS has also been used to assess metal concentrations in biological samples such as teeth. The method allows the analysis of the spatial distribution of trace elements in samples. [13, 286, 311] In the present study, LA-ICP-MS was used to measure lead levels in tooth samples in the epidemiological and histological study.

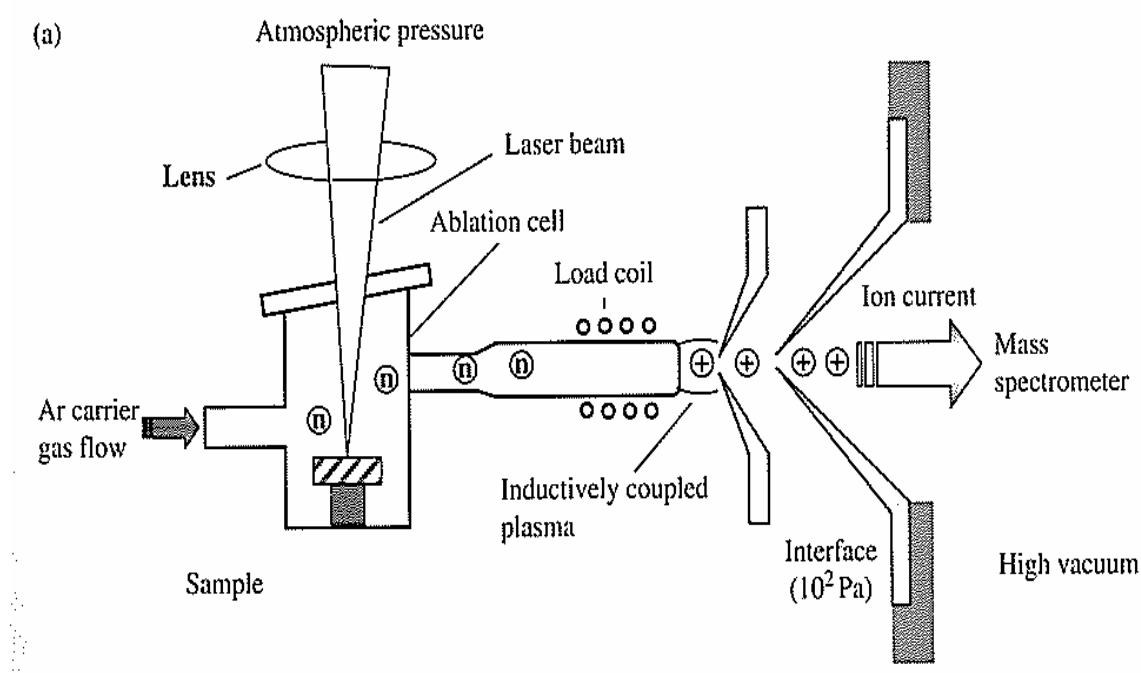
Some previous analysis of lead in human teeth using LA-ICP-MS has been undertaken. For example, this technique has been used to identify lead levels across sections of both ancient and modern human teeth. [238] Studies of the spatial distribution of lead in deciduous teeth by LA-ICP-MS have indicated that this method can be used for identifying trace element distribution in different regions of dental tissue which is calcified at different times of life. [13, 286] Recent studies used LA-ICP-MS in combination with tooth enamel histology in order to refine history of diet in children [245] and weaning stress in baboons. [246] Building on the techniques developed in these two studies, the LA-ICP-MS analysis was used in the present study.

#### **2.5.3.1 Principles and components of LA-ICP-MS**

The LA-ICP-MS technique requires the laser ablation of hard tissue samples with a delicately focused laser. Previously, Gray used a ruby laser type (wavelength 694nm) to vaporise solid samples before carrying the plasma to the ionisation process. [313] Since then other laser types, including CO<sub>2</sub>, N<sub>2</sub>, Excimer and Nd-YAG, have been used widely. [309] The Excimer laser used in the present study ablates at a wave length of 193 nm in an air-tight chamber and the originating aerosol plume is carried by an an argon plasma torch via a stream of argon gas where its constituents are atomized and ionized prior to

elemental mass analysis. The Excimer laser ablation, in particular, provides a thermal sample ablation with very precise lateral ablation and controlled depth profiling.

The introduction of LA coupled to ICP-MS has made it possible to perform the *in situ* element analyses of solid samples meaning time-specific data, such as that from a tooth surface, is able to be analysed. The LA-ICP-MS can reach detection limits of less than 1ppm for most elements levels, which can rarely be detected using other techniques such as the electron microscope or X-ray emission detector. Also, after analysis, the samples can be stored for future study. [286] The basic components of the LA-ICP-MS are shown in Figure 2.16



**Figure 2.16** LA-ICP-MS coupled with plasma source

From Becker, S. (2007) *Inorganic mass spectrometry: principles and applications*. Wiley: New Jersey [312]

### 2.5.3.2 Advantages and limitations of LA-ICP-MS

Currently, LA-ICP-MS is used to measure trace element distributions in teeth with precise, credible results. [5] Compared to previous studies using total digestion with acid followed by the analysis of solutions, the LA-ICP-MS allows the establishment of lead levels in each tooth component including the enamel surface, enamel-dentine junction, the neonatal line and other incremental structures of growth, the LA-ICP-MS output can also be mapped to explore the spatial multi-element distribution in solid samples at very

low detection limit (for lead from ppb). [13, 314] and it can therefore establish a ‘time line’ of lead exposure from in utero exposure through the early childhood years.

The drawback of LA-ICP-MS is the destructive nature of the ablation process. LA-ICP-MS cannot be used to map the spatial distribution of trace elements in soft tissues. [315]

# **CHAPTER THREE**

## **MATERIALS AND METHODS**

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## **MATERIALS AND METHODS**

### **3.1 Study design**

The aim of this study evolves from an investigation to explore the determinants of lead exposure using pre- and post-natal enamel and dentine to developing a method to assess the history of lead exposure by measuring tooth lead levels. Ultimately, this study was designed to answer questions about potential determinants of lead exposure and whether milk teeth are suitable biomarkers for revealing a time-line of exposure.

The study design consisted of three major phases. Phase I was an epidemiological investigation into the determinants of lead exposure as measured in dentine in a cohort of children from Newcastle upon Tyne. This involved the analysis of an existing collection of teeth from children living in Newcastle (the Tooth Fairy study) with questionnaire data available to identify potential determinants of lead exposure. Phase II examined the distribution of lead levels across the tooth surface. This was an exploratory study to assess the use of histological increments combined with tooth lead levels in dentine to reveal the history of lead exposure during the child's life-time. Phase III involved the application of this newly developed histological technique to a larger set of teeth to ascertain whether dentine is a suitable biomarker to determine a timeline of lead exposure. Participants from the Teesside areas in the North East of the UK were recruited for data collection in phases II and III of the present study.

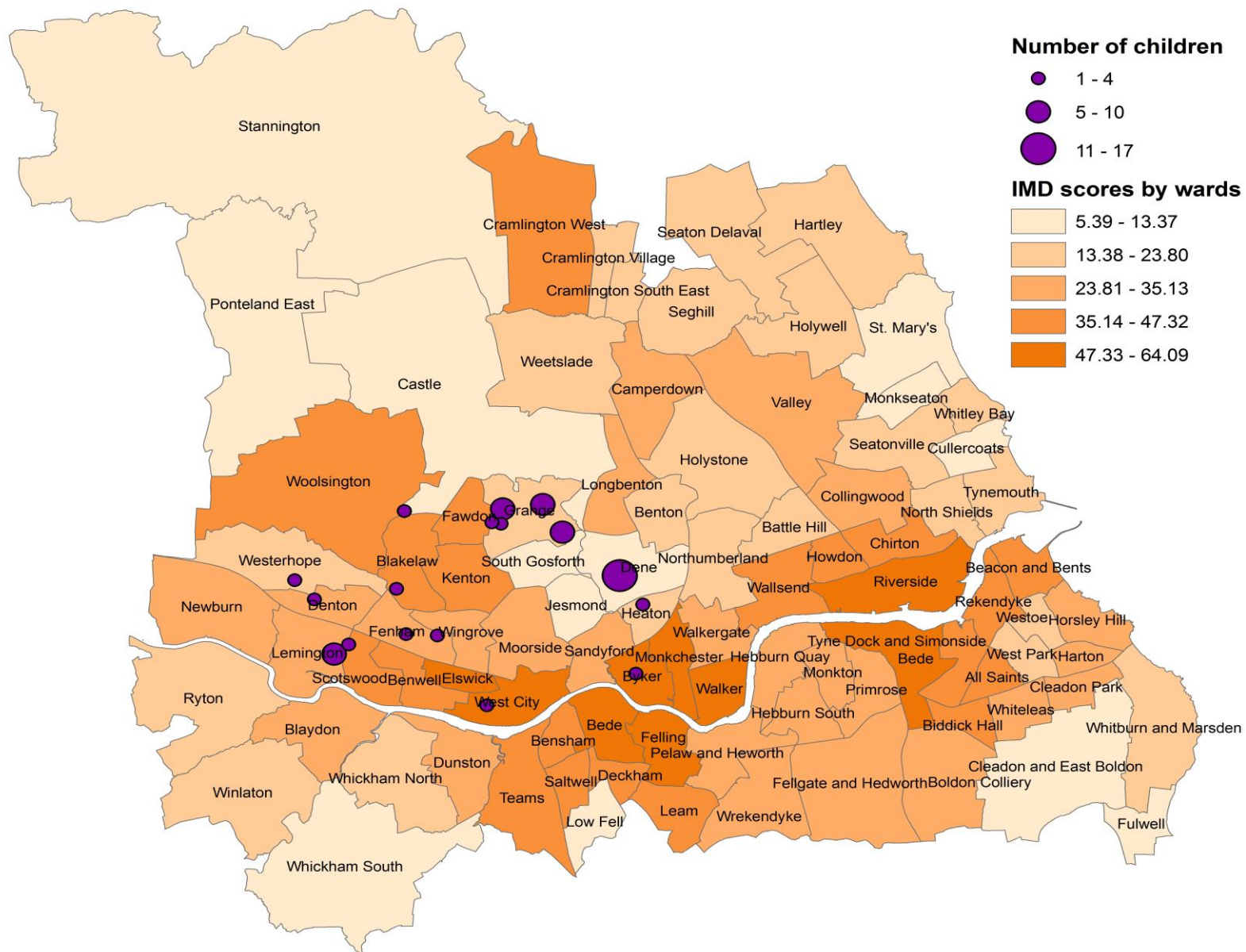
### **3.2 Phase I: The Tooth Fairy epidemiological study**

This epidemiological study involved the analysis of an existing collection of teeth ( $n = 69$ ) from the Newcastle Tooth Fairy study. In addition, existing questionnaire data were used to identify the determinants of lead exposure. The questionnaires are explained in detail in section 3.2.4.3.

### 3.2.1 Setting

Newcastle upon Tyne is located on the north bank of the river Tyne in north east England. Since the turn of the nineteenth century, the north east of England, and in particular Newcastle, has been a centre for heavy industry, and the region was one of the most intensely industrialised regions of the UK. [316] There is also a history of extensive lead mining within this area. A national survey conducted in 1981 and 1982 across the UK found lead levels in household dust and garden soils from 100 homes in Newcastle of around 600mg/kg and 300mg/kg respectively. [41, 317] A more recent study of soil contamination in Newcastle undertaken in 2003 showed that soil lead levels ranged from 40 to 4,134mg/kg (mean=350mg/kg). Of the 163 sampling locations, 17% (27 sites) exceeded the UK soil guideline value for residential areas and allotments of 450mg/kg. [151]

The population of Newcastle spans a wide range of social groups. According to data from Neighbourhood Statistics in 2009, the ethnicity of people living in Newcastle is made up of mostly white British ( $\geq 83\%$ ), Asian (7%), and white other (4.1%) residents, with remainder being black and mixed race. [318] Some of the most deprived communities in the UK live in Newcastle but also some of the wealthiest. The Index of Multiple Deprivation (IMD) Score is an area based measurement based on seven domains of deprivation: income, employment, health deprivation and disability, education skills and training, barriers to housing and services, crime and living environment. Each domain has a number of indicators. [319] Higher IMD scores indicate a more deprived area. When scored for multiple deprivation by council ward with a lower rank indicating higher deprivation, out of 8,414 wards in England and Wales, the ward of Byker ranked 78, Elswick 36, Monkchester 31, Walker 30 and West City 40. [319] Byker, Elswick, Monkchester and Walker are all located along the River Tyne, which was the major site of historically heavy industries. Currently, these are largely residential areas but historic contamination still persists. Conversely, Jesmond and South Gosforth wards were ranked 6941 and 7251 respectively, and historically few industries have been located in these areas. Although heavy industry in Newcastle has decreased drastically in the 20<sup>th</sup> century, many urban areas are likely to be impacted by historic environmental lead contamination. [319] Figure 3.1 shows Index of Multiple Deprivation (IMD) scores by ward across Newcastle highlighting the concentration of deprived areas alongside the River Tyne;



**Figure 3.1** Map showing locations of school divided by wards and IMD scores



### **3.2.2 Ethical approval**

The 2005 Tooth Fairy study was reviewed and approved by the County Durham and Darlington Local Research Ethics Committee (Reference Number: 05/Q0904/10).

Permission to use the data in this study was granted by Prof Tanja Pless-Mulloli, one of the main researchers involved in the study.

### **3.2.3 Selection criteria and sample size**

Children residing in Newcastle upon Tyne aged 5-8 years old who had lost an upper front incisor were eligible for inclusion in the sample. Children who had lived outside of the boundaries of Newcastle upon Tyne for more than one year, or those who were known to be moving within the next four months, those who had a history of lead poisoning and children from special needs schools were excluded from the study.

It was hoped that a total of 500 children would be recruited, since in order to detect a difference of 1.5ug/g in tooth lead between children in the least and most deprived quintiles of deprivation, (90% power, 5% significance level, 2-sided test) at least 80 children in each quintile was required. It was hoped that a extra 100 children could be sampled so as to ameliorate possible data attrition. However only 69 respondents met all of the criteria and answered the questionnaires during the proposed time. While this sample is much smaller than initially hoped, it was still considered worthwhile to explore the possible determinants of lead exposure in this smaller cohort, and despite not reaching the desired number of participants, this is still a relatively large cohort compared to others assessing lead levels in specific components of teeth.

### **3.2.4 Methods of data collection**

#### **3.2.4.1 Recruitment**

Initially a sample of 17 schools were randomly selected from a list of all primary or first schools stratified by a measure of the deprivation of the pupils attending the school. Three schools were selected at random from each of the five quintiles of school deprivation. For

each school, agreement was obtained from the headteacher and a covering letter and self completion questionnaires sent to the parents of all eligible children via the school. This letter described the study and asked the parents to sign a reply form if they were willing for their child to take part. If there was no reply then one reminder letter was sent out to parents. £10 vouchers were offered to children who had lost a front tooth and would provide that tooth along with the parental consent form and a questionnaire.

#### **3.2.4.2 Collection of sample teeth**

School nurses were trained to collect naturally shed milk teeth (upper incisors) from participating children. The nurses stored and labeled teeth samples and collected parental consent forms. Teeth were kept in sterile solution before being sent to the Hard Tissue Laboratory at the School of Dental Sciences, Newcastle University, for the preparation of tooth sections, cut and lapped to 300µm in thickness. Details of the standard protocol for sectioning and lapping tooth sections are given in Section 3.3.5.2 and 3.3.5.3

#### **3.2.4.3 Questionnaire**

The questionnaires consisted of 39 yes/no, true/false, single-best-answer, and some open-ended questions. The questionnaire was distributed to parents after being pre-tested and piloted. Four additional questionnaires were distributed to participants depending on their answers to the main questionnaire. All questionnaires are presented in Appendix I. Data from the questionnaires have been already entered into a Microsoft Access database and I exported the data into SPSS software for the purpose of statistical analysis. The main questionnaire for the Tooth Fairy study had five sections:

**Section 1:** Demographic data of participants including sex, date of birth, full residential history, and name and address of GP practice.

**Section 2:** Information about the child's current main place of residence including the postcode of the residence, any history of lead water pipes in the home, methods of cleaning floors in the house, frequency of vacuuming in the home, the age of the house (to determine if it was built before 1960 or later), and the existence and condition of lead in paint in the house.

**Section 3:** Child's eating and drinking behaviour was investigated. Questions covered the intake of allotment or home-grown foods, intake of tap drinking water, amount of tap water consumed daily, and breast feeding as well as weaning history.

**Section 4:** Other aspects of eating behaviour and health. Questions covered hand-to-mouth behaviour, intake of food stored in leaded storage containers such as pottery or ceramics glazed with lead glaze, lead crystal or pewter and lead soldered cans. Details of medical history including epilepsy, diabetes, asthma and either calcium or iron deficiencies were sought. In addition, pica behaviour was also investigated.

**Section 5:** This section asked about the educational level of the main wage earner in the family, the number of people in the household, parental occupation and income, smoking status, and the use of Asian cosmetics.

To assess socio-economic status, the tooth fairy study used the individual indicators of educational achievement and income of the main wage earner. In addition, the area based IMD Scores for 2007 of the Super Output Areas (SOA) that contained the child's home and school postcodes were explored. IMD scores for small geographical areas known as Lower Layer Super Output Areas (LSOA), a census based geography containing an average 1,500 residents, were assigned to each child based on the LSOA of their residential and school postcodes, with this linkage made using ArcView GIS 3.2 software. [320]

### **3.2.5 Data storage**

Tooth samples were stored securely in labelled vials until preparation in the Hard Tissue Laboratory in the School of Dental Sciences, Newcastle University. The storage and handling of the questionnaire dataset was undertaken using 'Microsoft Access 2003'.

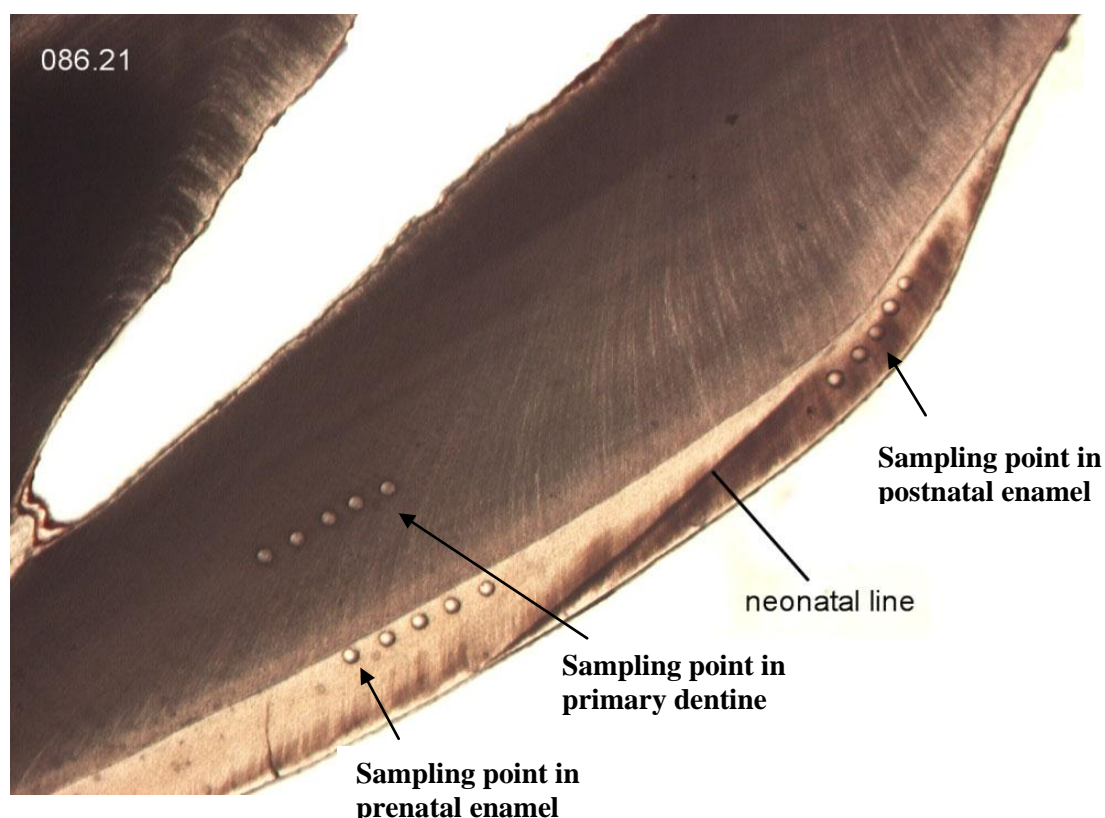
Seven databases were used as detailed below:

- (1) Dentine lead levels
- (2) Personal details from questionnaires and consent forms
- (3) Exposure data from questionnaires
- (4) Data linking ID codes and personal data
- (5) Details of children referred to clinicians

- (6) Data from linked databases to summarise the stages completed for each child
- (7) IMD scores of living areas and school areas

### 3.2.6 Analysis of the teeth

For each child recruited in the Tooth Fairy study, an upper deciduous incisor was collected once it exfoliated. To investigate lead levels in dentine, 4-6 ablation points were sampled in the dentine and enamel of each tooth. Tooth lead levels were analysed by LA-ICP-MS. Figure 3.2 illustrates the sampling ablation points in primary dentine, pre- and postnatal enamel from thick sections.



**Figure 3.2** Sampling ablation points on the tooth section in the Tooth Fairy Study

## **3.2.7 Statistical analyses**

### **3.2.7.1 Hypotheses**

The hypothesis explored in the epidemiological study was that there exists a relationship between determinants of lead exposure, primarily measured in terms of social class or socio-economic status, but also other lifestyle and home characteristics, and tooth lead levels.

### **3.2.7.2 Descriptive analyses**

Basic descriptive analyses, including frequency and percentage measures were used for an initial exploration of the data. Means and standard deviations were used to describe lead levels in dentine and IMD scores. A histogram was used to show the distribution of mean dentine lead levels. The Independent t-test was used to assess the relationships between dentine lead levels and dichotomised variables including sex and several questions with 'yes' or 'no' answers. Analysis of Variance (One-way ANOVA) was used to evaluate the relationships between lead levels in dentine and categorised variables including age, methods and frequency of cleaning hard floors in the house, tap water consumption and income. The Equal Variances Assumed using Scheffe method was used for post-hoc comparison. 95% confidence intervals were used to compare the mean lead levels in dentine between different variables.

### **3.2.7.3 Multivariate analyses**

To determine significant explanatory determinants of lead levels in dentine, multivariable linear regression analysis was used. A stepwise selection method was used to exclude or include determinants in a sequential process, with a statistical significance level of 95% ( $p < 0.05$ ) required for inclusion. All statistical analyses were carried out using Statistical Package for the Social Sciences (SPSS) version 17.

## **3.3 Phase II: Development of histological technique**

### **3.3.1 Introduction**

In the second phase of the study I aimed to develop a method to assess the use of tooth lead levels as a histological marker to reveal the history of lead exposure over a child's life-time. Although dental lead levels have been recognised to be a useful biomarker of lead exposure, limited information has been published on the distribution of lead in different parts of dental tissues. There is as yet no consensus on the relevance of lead levels from different parts of the tooth or on how these different levels relate to exposure. The mineralisation of the different tooth components is likely to be relevant, and determination of a suitable sampling strategy for teeth that are indicative of long term lead exposure in humans is crucial. Therefore, the present study was designed to test whether tooth lead concentrations could provide a non-invasive biological marker of the history of lead exposure in children. Such a marker would have useful applications in epidemiology and public health.

### **3.3.2 Participant recruitment and selection criteria**

Participants were recruited from the Queensway Dental Practice at Billingham, a small town in the Borough of Stockton on Tees in north-east England. Fifteen children aged between 6 and 8 were recruited into this study. All had been identified as needing to have two deciduous molars removed for dental hygiene. This was a pilot study of 15 children, and as such it was difficult to undertake a formal power calculation. Written consent was provided by the parents of all participants. Children or their parents who did not provide consent were not eligible, and children whose teeth were found upon removal to be very carious were excluded from the analysis.

### **3.3.3 Ethical approval**

Phase II and III of this study involved working with human teeth and blood, and the protocol was therefore submitted and approved by the following ethics committees:

1. County Durham & Tees Valley Primary Care Trusts, Research and Development, who approved the research on 22<sup>nd</sup> May 2009 (local R&D number: 0405)
2. National Research Ethics Service (County Durham & Tees Valley Research Ethics Committee (REC)) who approved the research on 10<sup>th</sup> July 2009 (REC reference number: 09/H0905/42).

The main ethical issues involved consent, confidentiality and data storage. The details of each issue are outlined below:

### **3.3.3.1 Consent**

The children recruited into this research study were of a very young age, and unlikely to be able to provide informed consent about their participation. I explained to each child (with their parent/guardian present) what the study was about, and what their participation involved, using language suitable to the age group concerned. However, it was ultimately the decision of the parent/guardian as to whether or not their child participated. If the parents or guardians chose that their children should take part in the study, they were asked to sign the consent form provided. (see Appendix II)

### **3.3.3.2 Confidentiality and data storage**

The personal data collected for this research includes the age and sex of the participants. These data were stored only on the hard copy of the consent forms, which remain locked in a cabinet. The participants were assigned a numeric code which linked their personal details to their blood lead and tooth lead levels. These data were uploaded and analysed using a password-protected university desktop computer. The code key linking the ID code to personal identifiers were known only to the dental clinic, and contact details for the participants were required only in the unlikely situation that the blood lead levels measured were higher than the WHO threshold for health (10µg/dL), in which case the participant's GP would be informed.

### 3.3.4 The process of the collection of teeth and blood samples

For selection purposes, eligible participants identified by the dental team were interviewed for approximately 5-10 minutes each in order to understand the importance of the research and what was involved. There was a period lasting days or weeks between the initial check-up dental appointment and follow up surgery, during which the participant could decide on whether or not to take part in the study. During this time they had opportunity to contact the research team to ask questions about their participation. If children were willing to donate their milk teeth, they brought the completed consent form with them to the next scheduled dental appointment.

The consent forms were collected from willing participants by a member of the research/dental team during the follow-up appointment and/or the day of surgery to remove the teeth. From the consent form, the patient's ID was coded and matched with the patient details at the dental clinic. Two copies of the consent form were kept separately; one by the child and one by the researcher.

During the process of tooth extraction, the dentists checked the patient's details. If they matched with the coded data in this study, the two extracted deciduous molars were kept in a container provided by the research team. The patient number was labelled on the container. After extraction, the teeth were washed in distilled water by nurses and stored in 5% Chloramine-T solution, used to maintain the stability of biological tissues and keep the teeth sterile, and also known not to contain ingredients that might interfere with the ablation process. The participant's sex, age and the date of extraction were marked on the container. These containers were kept in a refrigerator at 4°C and subsequently brought to the Hard Tissue Laboratory in the School of Dental Sciences, Newcastle University.

A 5ml blood sample was collected via the indwelling cannula used to deliver the sedative during the dental work. These blood samples were split into 2 fractions (3.0ml and 2.0ml) and were stored separately as follows:

(1) The 3.0ml aliquot was stored in a clean 'blank' polypropylene tube labelled **NO ANTICOAGULANT**, with the patient number on the tube.



(2) The 2.0ml aliquot was stored in a polypropylene tube labelled **LI HEPARIN** printed on an orange bordered label and with the patient number on the tube.

These second tubes were inverted 6 to 8 times to mix the blood with the heparin. Both tubes were kept in a refrigerator. The first was used for lead isotope analysis by Dr Tom Shepherd, and the second being sent to the Health and Safety laboratory in Buxton for analysis.

### **3.3.5 Preparation of teeth for the histological study**

Teeth were sectioned, polished and mounted onto a slide in preparation for LA-ICP-MS and histological analysis. For each tooth/section, this process took approximately 4-5 hours to complete. Fifteen children in the histological study were assigned hard tissue numbers (HT) from HT1 to HT15 and all these children had measured BLLs. Of the 30 teeth (15 pairs), paired teeth from four children (HT4, 7, 10 and 15) and one tooth from child HT6 (tooth 65) were excluded from the study due to caries and attrition. As a result, only 21 teeth were analysed for tooth lead levels. Of these 21 teeth, one tooth from child HT6 (tooth 74) and child HT8 (tooth 84) were subsequently excluded from the histological study because it was not possible to identify growth increments across the tooth surface due to poor section quality. As such, 19 teeth were included in the histological study. Two pairs of teeth from child HT1 (tooth 54 and 55) and HT11 (tooth 64 and 85) were selected to develop the histological technique in the second phase, and the 15 remaining teeth were then used in the third phase of the present study.

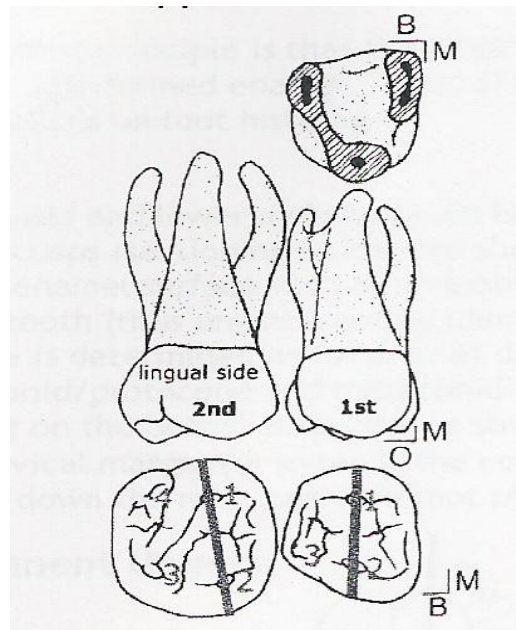
The preparation of sample teeth included the following processes:

### **3.3.5.1 Tooth catalogue**

The 21 teeth were examined and catalogued to identify tooth type; details of tooth types and orientation are given in Table 3.1. Tooth codes refer to specific teeth in accordance with the 2 digits derived from the Federation Dentaire Internationale (FDI) method, which identifies and designates permanent and deciduous teeth within the oral cavity. (see Section 2.3.1)

### **3.3.5.2 Tooth sectioning**

The stored deciduous molars were rinsed in tap water for 2 hours in order to remove the Chloramine-T, and then air-dried overnight. Teeth were marked for sectioning. Permanent red felt-tip pen was used to mark the point of the cusp tips, make a line to identify the section to be cut. The mesiolingual cusp was preferred as the area of interest because it is the first cusp of the tooth to develop. The ideal plane of section for a crown should always include the first and last formed enamel because they can provide information on the chronological development from the point of crown eruption to the completion of the root. Due to caries and attrition, distal sections were also cut and all teeth were cut near the tips of the cusps as well as passed on the distolingual side. To achieve thin sections, the tooth was attached to the chuck with sticky wax, which was heated using a Bunsen Burner. The teeth were then sectioned longitudinally with a low speed saw (Microslice Metals Machine) with a 250µm thick blade. Sections 500 µm thick were taken. Figure 3.4 shows the desired plane of sectioning for an upper deciduous molar. M and B refer to Mesial and Buccal cusps, and O to Occlusal view. For the tooth pairs from each child, I was not always able to use the same tooth cusp due to tooth caries. Section orientation for each tooth is provided in Table 3.1, and the desired plane of sectioning for upper deciduous molar in this study is given in Figure 3.3.



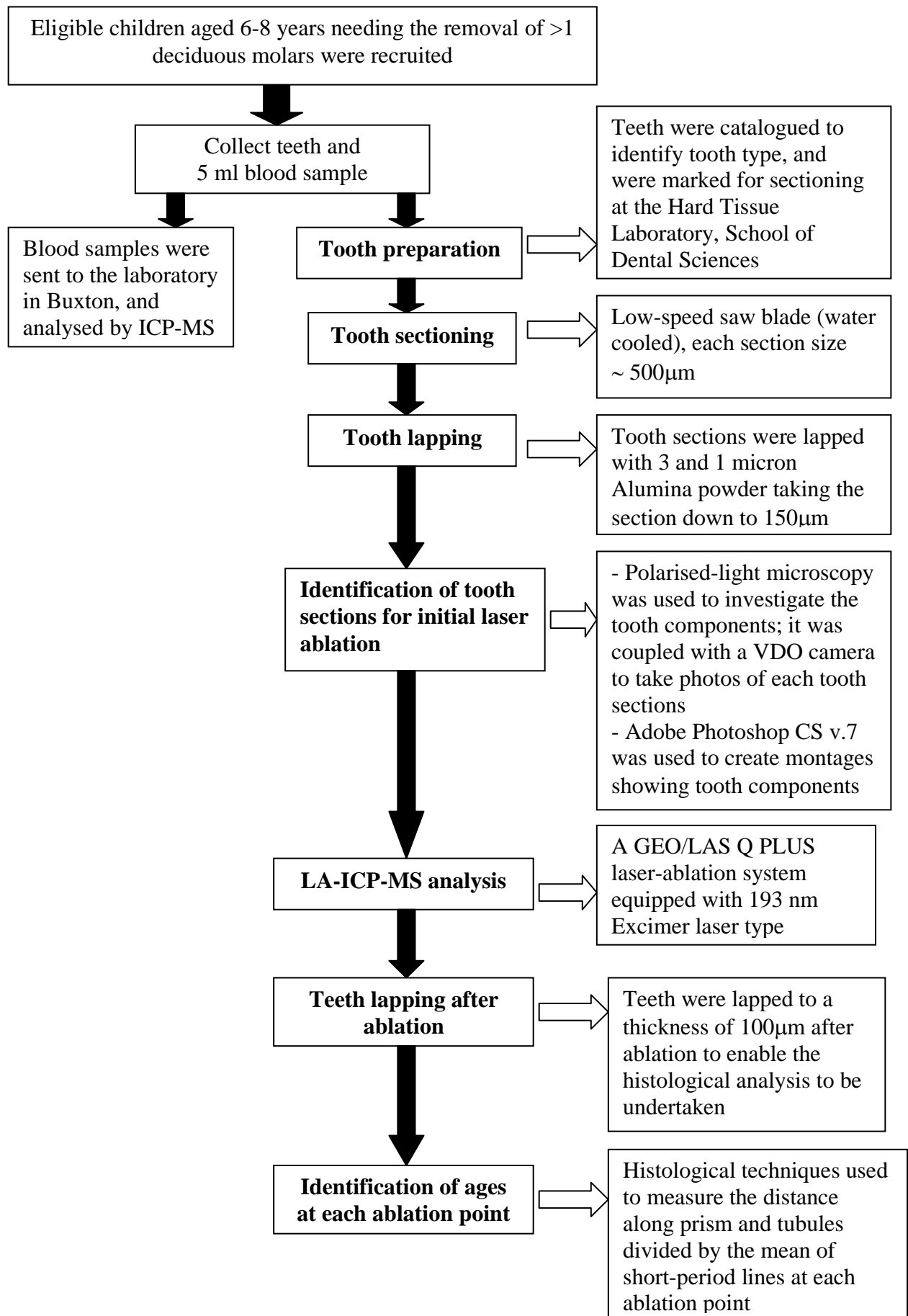
**Figure 3.3** The desired plane of sectioning for upper deciduous molar [Picture courtesy of Pam Walton]

**Table 3.1** Characteristics of tooth samples in the histological study

No	Hard Tissue Number	Tooth types	Toothcodes	Section orientation
1	HT1	Maxillary right first molar	54	Mesial
		Maxillary right second molar	55	Distal
2	HT2	Maxillary right first molar	54	Mesial
		Maxillary left first molar	64	Mesial
3	HT3	Maxillary right second molar	55	Distal
		Maxillary left second molar	65	Mesial
4	HT5	Maxillary right first molar	54	Mesial
		Maxillary left second molar	65	Distal
5	HT6	Maxillary left second molar	65	Mesial
		Mandibular left first molar	74	Mesial
6	HT8	Mandibular right first molar	84	Distal
		Mandibular right second molar	85	Distal
7	HT9	Maxillary right second molar	55	Distal
		Maxillary left second molar	65	Distal
8	HT11	Maxillary left first molar	64	Mesial
		Mandibular right second molar	85	Distal
9	HT12	Mandibular right first molar	84	Mesial
		Mandibular right second molar	85	Mesial
10	HT13	Mandibular left second molar	75	Mesial
		Mandibular right first molar	84	Mesial
11	HT14	Mandibular left first molar	74	Mesial
		Mandibular right first molar	84	Mesial

### 3.3.5.3 Tooth lapping

After the ~500µm section was cut, the required surface was lapped down first. The section was adhered to a microscope slide with sticky wax for lapping to the required thickness of 150µm. This thickness was sufficient to allow the structure of the tooth to be identified under light microscopy to identify transect locations for LA-ICP-MS, while being sufficiently deep for the ablation process. The surface of each section was polished with 3 micron alumina powder using a PM2A lapping machine (Logitech Materials Technologists Engineers). The slide was consequently polished with 1 micron alumina powder to make a smooth, scratch-free surface with no saw marks, then washed with ecological washing-up liquid to remove grease and wax, and subsequently cleaned in an ultrasonic bath. The tooth slide was air-dried over night and then zero bonded to a clean glass slide. Xylene and UV resin glue were then used to fix the tooth section to the slide. In the final lapping process, the thick section was lapped by hand using polishing paper. The slide was then polished with 3 micron alumina powder and the tooth thickness was checked with a Micrometre Digimatic (Mitutoyo, Manual No. 1038, Serie N° 293). The finished slide was then cleaned in an ultrasonic bath to remove surface lapping compound and washed in distilled water and left to dry. Once each section had been ablated using LA-ICP-MS, it was lapped down further to 100µm using 1 micron alumina powder. The 100µm sections enabled the study of micro structures within the enamel and dentine via microscopy, and the identification of the neonatal line which is not clearly visible in thicker sections. Figure 3.4 shows a flow diagram of the sample collection, preparation and analyses and Figure 3.5 illustrates the preparation of the sample teeth.



**Figure 3.4** A flow diagram of the sample collection, preparation and analyses



**The desired plane for sectioning**



**Tooth sectioning**



**Slice prepared for lapping**



**Tooth lapping**

**Figure 3.5** The preparation of the sample teeth

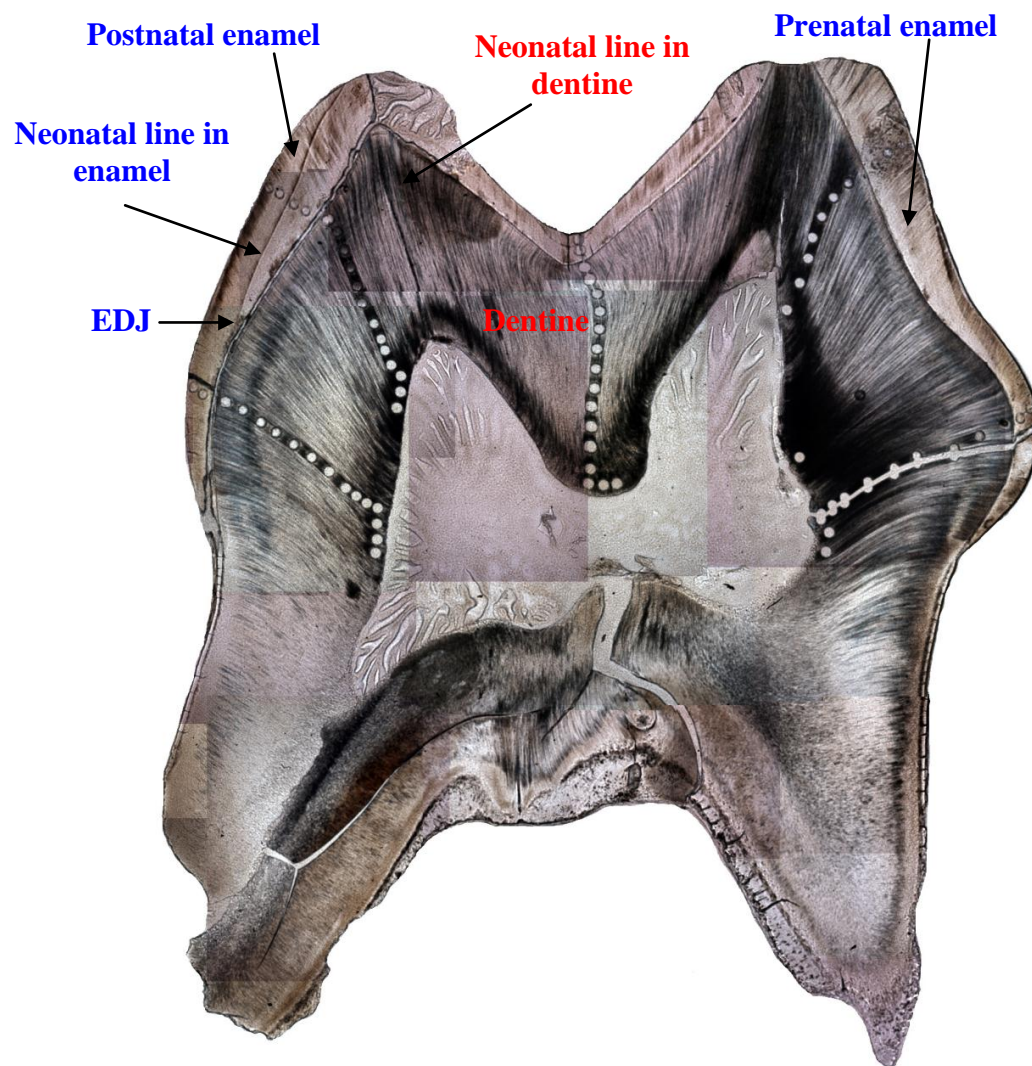
### **3.3.6 Identification of sections for initial laser ablation**

To pilot the techniques, four sections were chosen to be ablated along 5-6 transects per tooth section. These four teeth were chosen from 2 children, child HT1 (teeth 54 and 55), and child HT11 (teeth 64 and 85) as the child HT1 had high BLL and no carious teeth and child HT11 had the lowest blood lead levels. The procedure used to identify the region of prenatal and postnatal enamel, and dentine in deciduous molar is described in this section.

An Olympus BX51 polarising microscope was used to investigate each ground section. The neonatal line was recorded in each tooth section, and was used to establish a baseline of sampling points within the tooth enamel and dentine.

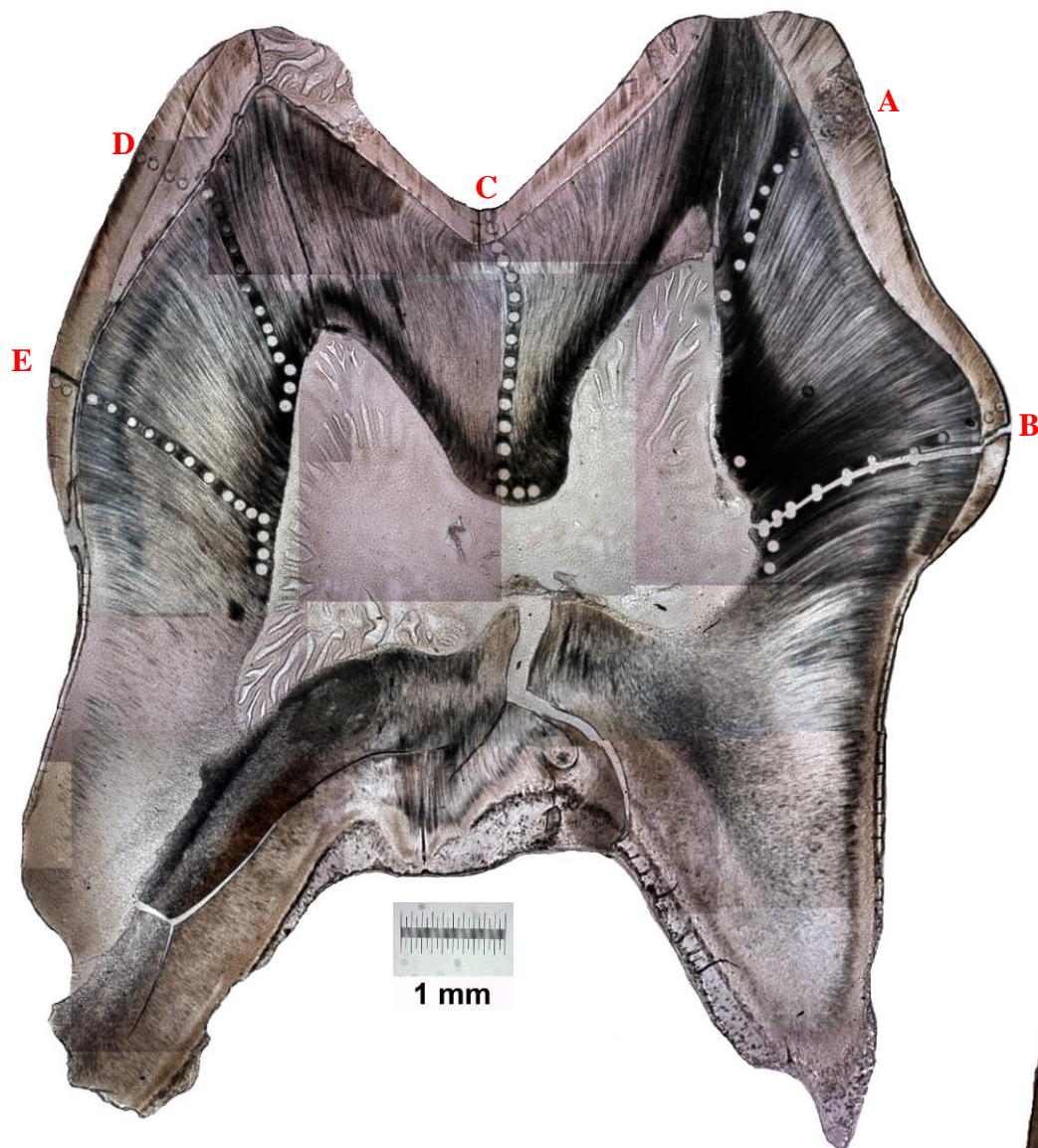
To investigate lead levels in enamel, discrete sampling points were ablated along the prism trajectory running from the EDJ to the enamel surface. I used the neonatal line as a fixed point of birth. It is easier to find the neonatal line in enamel and I started there, and divided pre- and postnatal enamel. The intersection at the EDJ was then used to identify the neonatal line in dentine; and from that pre- and postnatal dentine were identified. Figure 3.6 shows the incremental lines in enamel and dentine from a mesial section of an upper first deciduous molar.

For the initial four teeth I identified transects for ablation that would include pre- and postnatal enamel and dentine across the tooth surface. For child HT1, in each section (HT1-54 and HT1-55) I identified five transects (A-E) for laser ablation. For child HT11, six transects were identified (A-F) for HT11-64, while five transects (A-E) were identified for HT11-85. Figure 3.7 and 3.8 illustrate the ablation points for the initial four teeth.

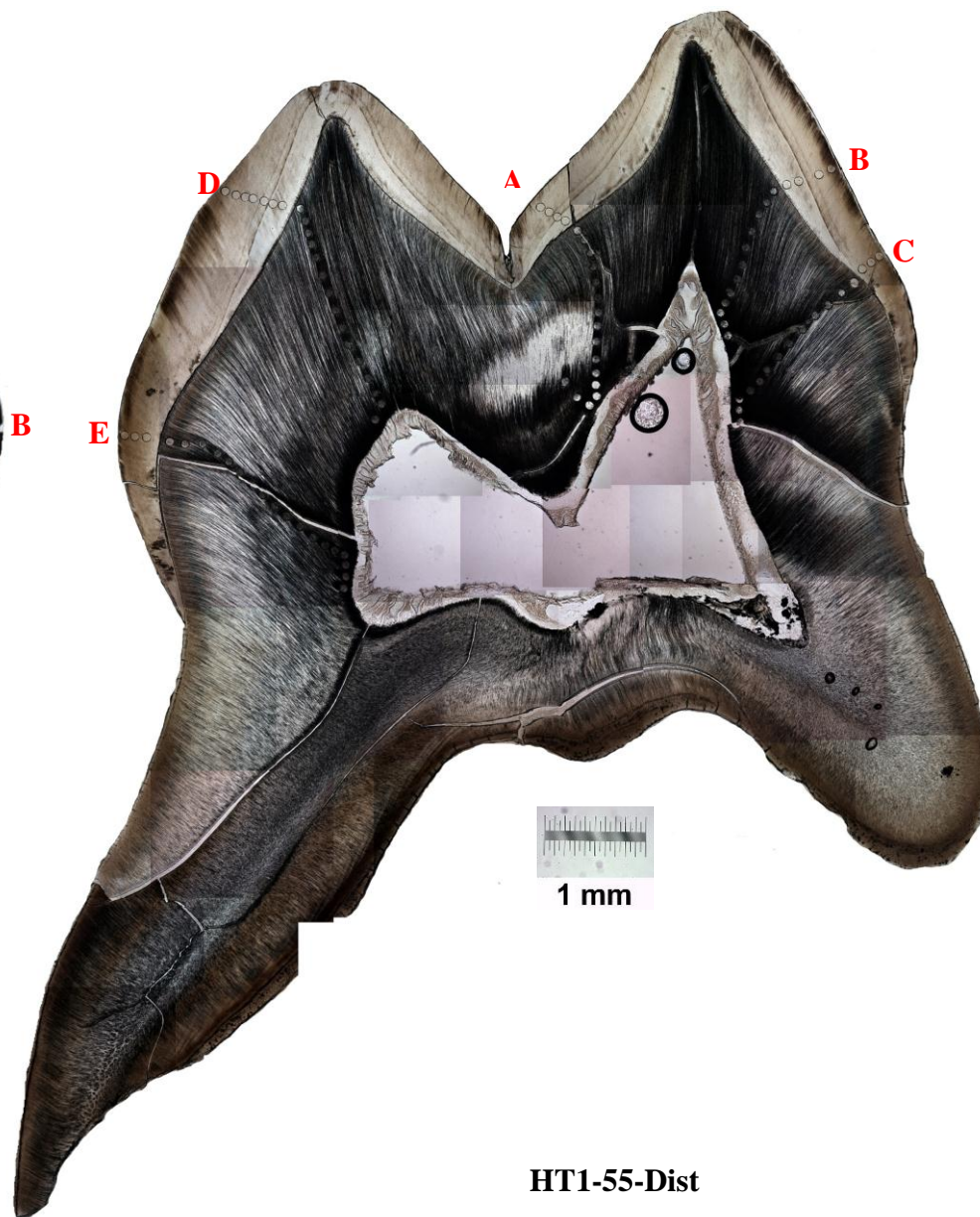


**Figure 3.6** Pattern of incremental lines in enamel and dentine





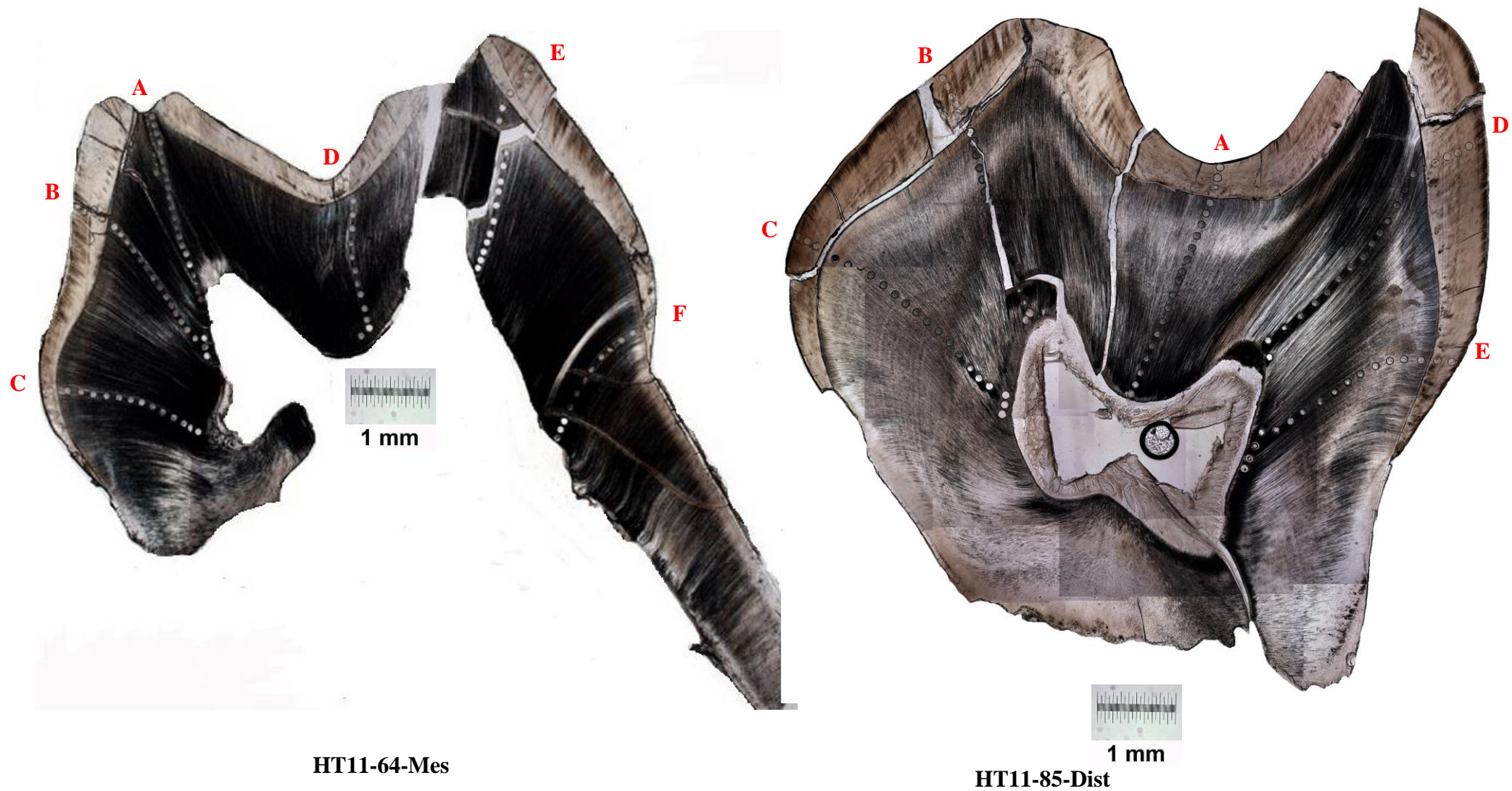
HT1-54-Mes



HT1-55-Dist

**Figure 3.7** Ablation transects for the two tooth sections from child HT1





**Figure 3.8** Ablation transects for the two tooth sections from child HT11

### 3.3.7 LA-ICP-MS analysis of the sample teeth

The distribution of calcium normalised lead ratios ( $^{208}\text{Pb}:^{44}\text{Ca}$ ) referred to here as lead ratios, in the different growth layers were quantified in tooth sections by LA-ICP-MS. The principle of LA-ICP-MS are described in detail in chapter 2. The tooth samples were analysed at the University of Leeds (School of Earth and Environment) using a GeoLas 193nm ArF excimer laser coupled to an Agilent 7500c ICP-mass spectrometer. This configuration permits high precision, multi-element analysis to be performed on very small samples at high spatial resolution.

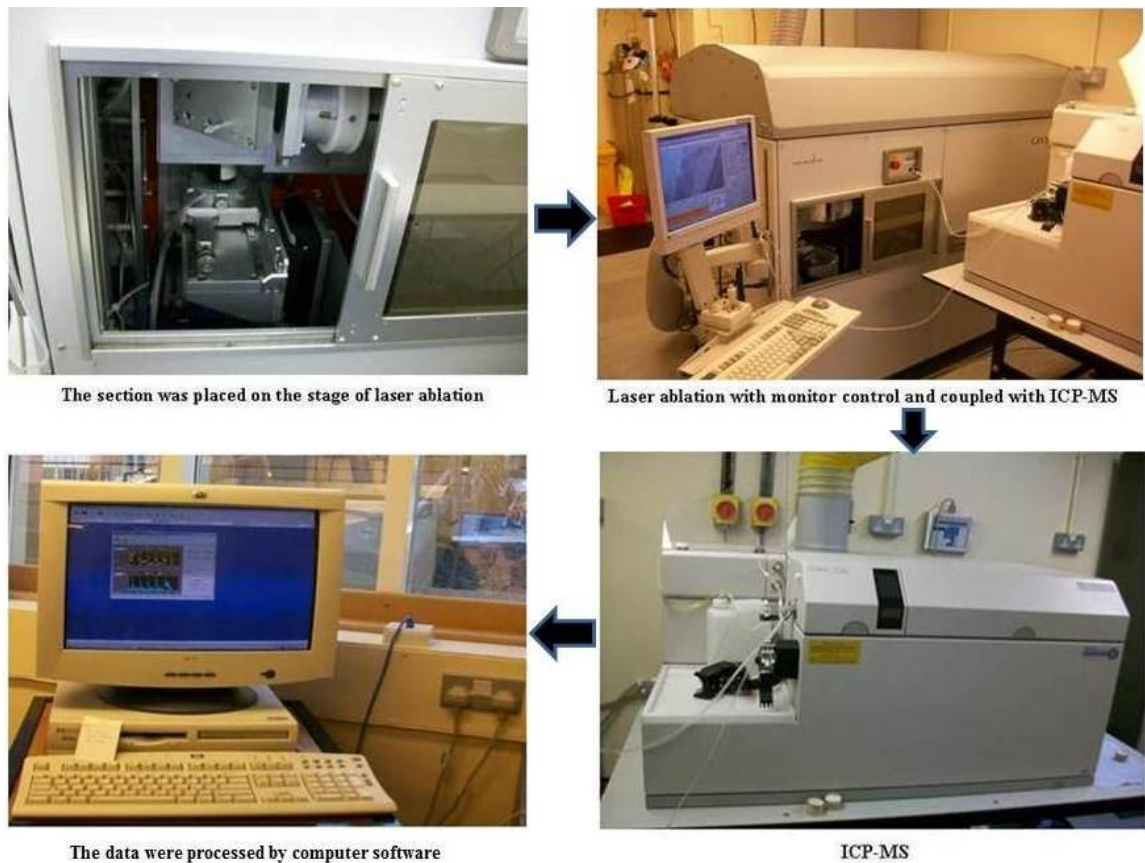
For the analysis of the tooth samples the most critical laser parameters are given in Table 3.2. For the ICP-MS instrumentation, intelligent auto-tuning software was used to ensure optimum and consistent daily operating conditions. Lead determinations were made in time resolved scanning mode using  $^{208}\text{Pb}$  normalised to  $^{44}\text{Ca}$  to adjust for within-sample variation in calcium and/or changes in ablation rates. No spectral interferences for either isotope was noted. The reference material NIST SRM Glass 610 ( $426\mu\text{g/gPb}$ ) was used for instrument calibration and cross-referenced to replicate the analyses of NIST SRM Glass 614 ( $2.32\mu\text{g/gPb}$ ) and NIST SRM Bone Meal 486 ( $1.34\mu\text{g/gPb}$ ) before and after each analytical session in order to validate instrument performance and within-run standard errors. Minimum detection limits were calculated as 3 times the background count rates on the carrier gas blank before ablation. Typical minimum detection limits for lead in dentine and enamel were  $0.005\text{--}0.03\mu\text{g/gPb}$ . Relative changes in lead were expressed as Pb/Ca wt% ratios. Absolute concentrations of lead were estimated assuming a known concentration of calcium in the sample of  $374,000\text{ppmCa}$  for enamel and  $265,000\text{ppmCa}$  for dentine. [13] Data processing was performed off-line using SILLIS; a software programme specifically written for the signal integration of laboratory laser systems by Murray Allan (University of Leeds) and subsequently modified by Dimitri Meier and Marcel Guillong (Die Eidgenossische Technische Hochschule, Zurich). Table 3.2 provides laser operation parameters and related ICP-MS conditions.

**Table 3.2** Laser operating parameters and related ICP-MS conditions

<b>Laser ablation operating conditions</b>	<b>Values</b>
Laser energy	10J/cm <sup>2</sup>
Laser pulse rate	5Hz
Pulses per ablation analysis	200
Beam diameter	100µm
Background acquisition time	~20secs
Signal Acquisition time	~40secs

A series of 100µm diameter ablation pits were made at intervals of 100-200µm from the outer enamel surface, across the enamel/dentine junction to the dentine/pulp cavity interface along pre-identified transects. During ablation, the process was continuously monitored on a computer screen via a video camera and visible light sources integrated into the optical array.

The LA-ICP-MS recorded elemental intensities were processed using the SILLS software package [321], which was originally developed in Leeds. Data were then imported into Microsoft Excel 2007. Lead ratios are multiplied by 10<sup>7</sup> through this thesis. Figure 3.9 shows the main components of the LA-ICP-MS used in this study.



**Figure 3.9** The laser ablation was coupled with the ICP-MS

### 3.3.8 Age measurement at each ablation point

After the initial four teeth were ablated, they were brought back to the Hard Tissue Laboratory, School of Dental Sciences, Newcastle University, in order to undertake the histological analysis. Teeth were lapped down another 50 $\mu$ m. The tooth sections were turned over so the ablated side was on the slide and then lapped in from the side that was not ablated. The purpose of this was to ensure that the ablation pits did not get lapped away, so that they can be related to the daily growth increments. The estimation of the age of each ablation pit comprises the following three steps: 1) the identification of neonatal line in enamel and dentine, 2) the measurement of distance along the prism in enamel and along the dentinal tubule in dentine, and 3) deriving ages by dividing the distance by mean daily secretion rates. The following section describes the histological technique developed to measure age at each ablation point in enamel and dentine.

After determining the position of the neonatal line in enamel, directions and distances to the ablation pit along the prism in enamel were measured. The mean daily rate of enamel

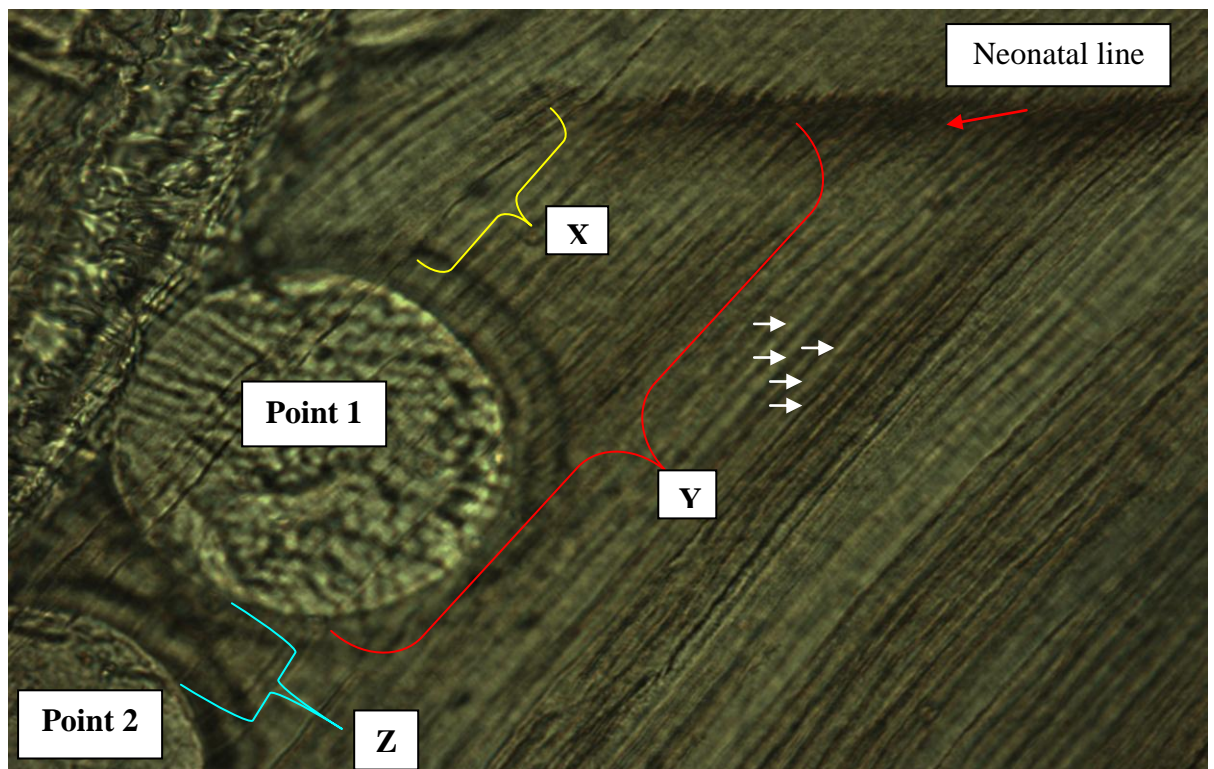
secretion was determined by the mean distance between the daily growth increments (the cross-striations in enamel). The age for each ablation point was calculated by dividing the distance ( $\mu\text{m}$ ) from the neonatal line with the mean daily growth rate. This gave the number of days and the age from birth to the edge of the enamel sampled by the pit. The diameter of the ablation pits was constant at  $100\mu\text{m}$ . The mean rate of enamel secretion was determined alongside the pit to yield the number of days sampled by each pit and the age at the end of the sample. In a similar manner, the age of the next pit was determined by measuring the distance from the edge of one pit to the next, calculating the mean secretion rate of enamel between the two and dividing to yield the number of days between the samples. This was added to the age at the end of the previous ablation pit to give the age at the onset of the next sample. The process was continued until the ages sampled by each pit were determined. A series of age ranges of six weeks were constructed to encompass the maximum number of days within an ablation pit and the elemental data from each ablation pit were assigned to one of these age ranges.

A similar strategy was followed to determine the ages sampled by each ablation pit in the dentine, using the daily short-period lines known as von Ebner lines. The position of the neonatal line in dentine was determined; the distances along the dentinal tubule in dentine were also measured. The distance was divided by the mean daily rate of dentine secretion as determined by the mean distance between the von Ebner lines. [269] The age for each ablation point was calculated by dividing the distance ( $\mu\text{m}$ ) with the mean daily growth rate. This gave the number of days and the age from birth to the edge of the dentine sampled by the pit. As in enamel, the size of the ablation pits was constant. The mean rate of dentine secretion was determined alongside the pit to yield the number of days sampled by each pit and the age at the end of the sample. In a similar manner, the age of the next pit was determined by measuring the distance from the edge of one pit to the next, calculating the mean secretion rate of dentine between the two and dividing to yield the number of days between the samples. This was added to the age at the end of the previous ablation pit to give the age at the onset of the next sample. The process was continued until the ages sampled by each pit were determined. As with enamel, age ranges of six weeks were used to encompass the maximum number of days within an ablation pit and the elemental data from each ablation pit were assigned to one of these age ranges.

For the most part, the histological sections in the present study showed clear incremental lines in both enamel and dentine. To determine the ages and duration of time sampled for the ablation pits, measurements followed along transects where incremental lines were easy to identify. Previous studies of incremental features in deciduous teeth have found them difficult to identify clearly and few studies have been made of primary teeth. Fitzgerald and Saunders (2005) suggested overcoming this problem using a practical analytical method. That guideline uses information from tooth structures that are clearly distinguished, and to interpolate or extrapolate in other tooth areas. For example, when cross striations are difficult to visualise in primary tooth enamel, the total time for prism growth is measured based on repeatedly calculated cross-striation intervals at any points where these striations are clearly seen, as close to prism as possible. [268] Goodman and Rose (1990) tested incremental lines could be matched chronology of pairs teeth from the same child and set criterion that the accentuate should be visible almost 75% of its length from enamel dentine junction to the tooth surface [322], which Dean and Scandrett (1995) then applied and proposed an approach to identify difficult incremental lines in each tooth region, for instance, the number of clearly identifiable cross striations per 1 cm on each montage was counted repeatedly for each enamel regions (inner, middle, outer). [279] This strategy was also applied to the present study in a few sections where cross-striations were difficult to identify. In dentine, incremental lines were observed more clearly and it was not difficult to measuring age or duration of each ablation pit in dentine.

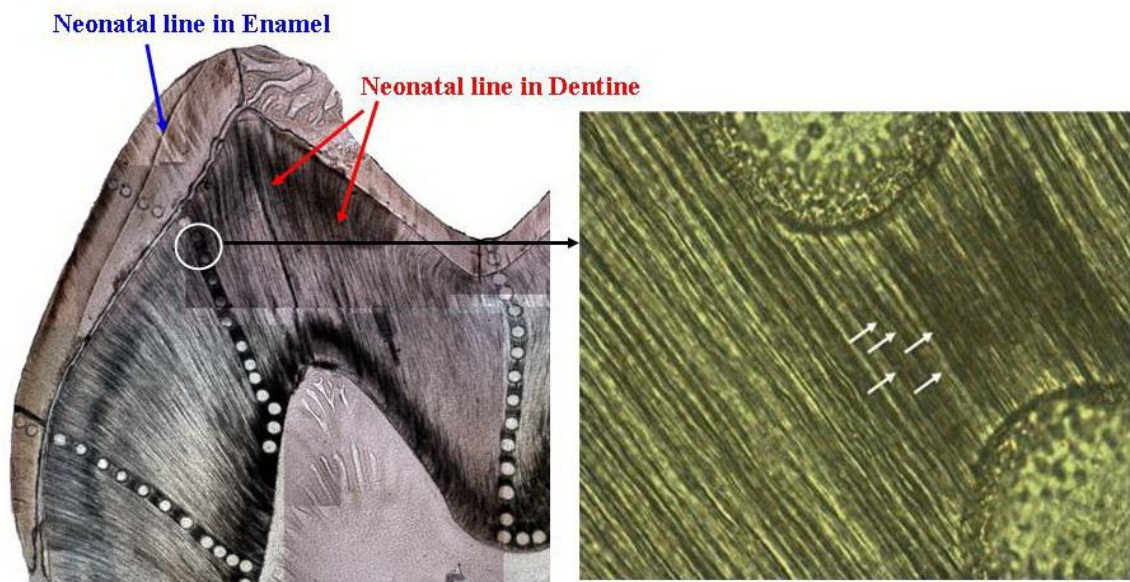
Figure 3.10 shows an estimation of the age of an ablation point in postnatal enamel. To estimate the age at the point adjacent to the neonatal line, the distance from the neonatal line to the near and far edges of point 1 were measured and identified as X and Y. Then, ten cross striations (white arrows) were measured and the mean daily secretion rate was derived. Age was then calculated by dividing distance of either X or Y by the mean of the cross-striations. Next, to estimate the ages between two ablation points, the distance between two points (1+2) and was measured as Z. Cross striations between those points were then measured, and the mean daily secretion rate was derived to calculate the age between these two points. This method was used to measure the age of each subsequent point across the enamel surface. The cumulative age from the neonatal line to the far edge of the last point would represent the sum of the individual ages including the age between points.





**Figure 3.10** Age measurement at ablation point in postnatal enamel

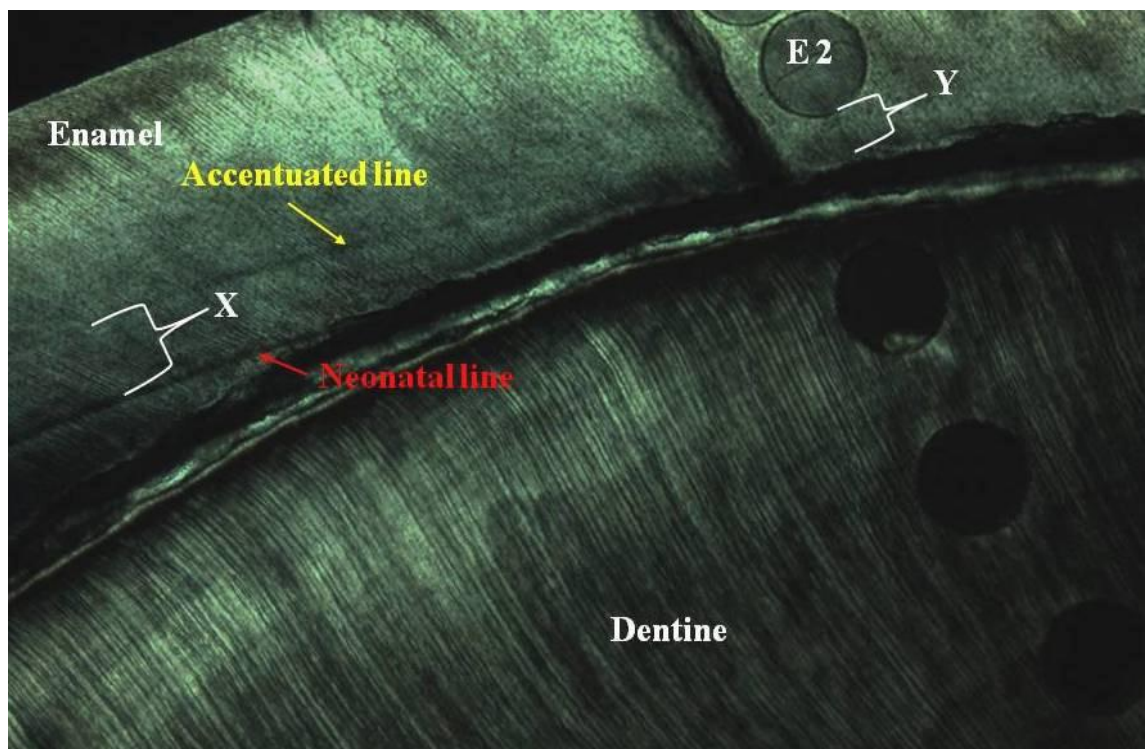
This technique was also used for dentine. The neonatal line was used to divide pre-natal and post-natal dentine. Figure 3.11 presents an identification of the neonatal line in dentine and the white arrows show the direction of von Ebner's lines in primary dentine.



**Figure 3.11** Age measurement at ablation point in primary dentine



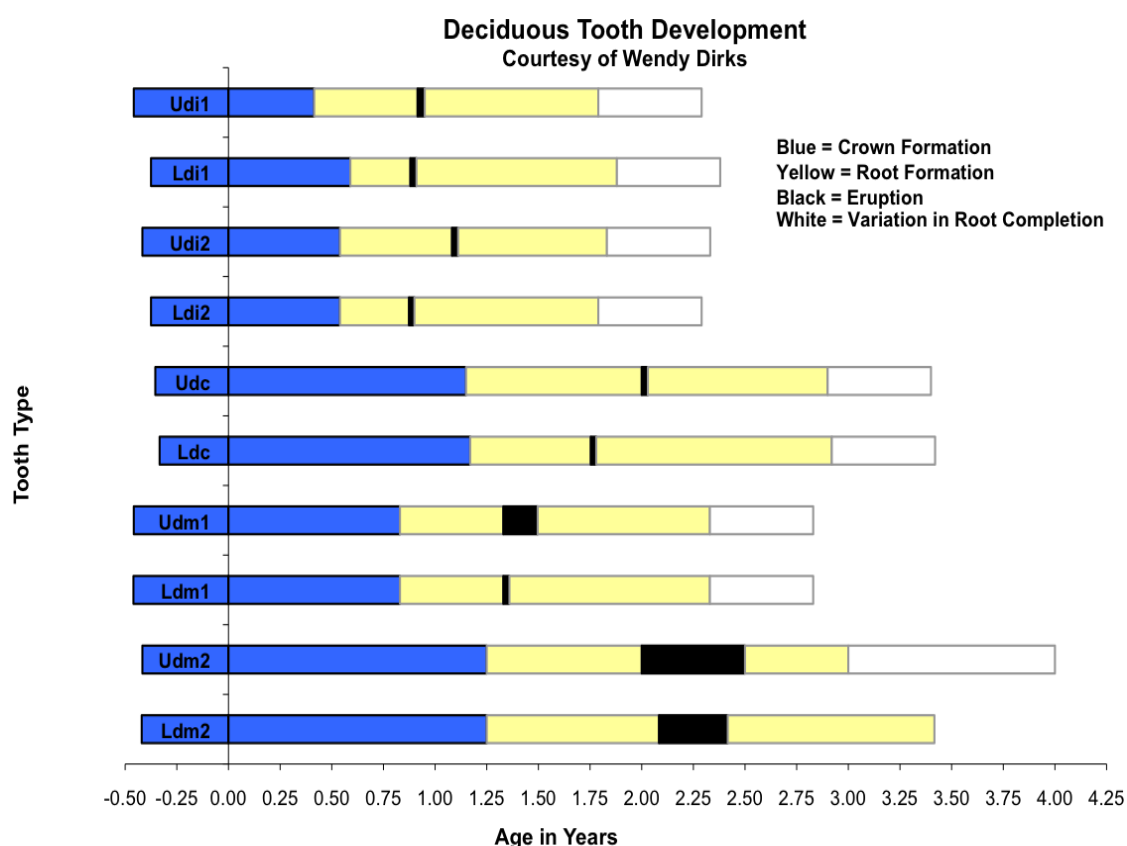
The neonatal line was determined in most of the tooth sections; however, the ablation points in some sections did not sample across the neonatal lines (shown as point E2 in Figure 3.12), and the method described above was therefore not suitable to measure the age of the tooth at that point. An alternative technique using other prominent accentuated lines was used to determine the age at these sampling points, which is illustrated in detail in Figure 3.12. Firstly, the distance from the neonatal line to the prominent accentuated lines was marked as X. The number of daily cross-striations was measured using the same technique described above, which was used to calculate the average daily secretion rate. Subsequently, the distance between this accentuated line to the edge of the ablation point E2 was measured as Y, and divided by the daily secretion rate to determine the number of days between the lines. Since the neonatal line identifies birth, the accentuated lines provide an age at tooth initiation arrived at by adding the number of days from birth to the other subsequently accentuated lines. Therefore, the number of cross-striations is measured from any accentuated line of known age to the edge of an ablation point nearest to the EDJ. For example, the age of the ablation point sampled across E2 is 30 days, if an age of 70 days is calculated from the neonatal line to the accentuated line ( $X=70$  days) and the age from the accentuated line to the nearest edge of E2 is 20 days ( $Y=20$  days), the age from the neonatal line to the near edge of the ablation point E2 is 90 days ( $70+20$  days), and the age across point E2 is 90-120 days ( $90+30$  days).



**Figure 3.12** Age measurement using accentuated lines in postnatal enamel

In the present study, the distance between cross striations measured in enamel for the initial four teeth ranged from 3.34-3.94 $\mu$ m (mean $\pm$ SD=3.6 $\pm$ 0.3). In dentine, the distance between von Ebner or short-period incremental lines were between 2.75 $\mu$ m and 2.96 $\mu$ m (mean $\pm$ SD=2.9 $\pm$ 0.1).

In the initial four teeth, the number of days within each ablation pit in enamel ranged from 22-32 days (Mean $\pm$ SD=27.6 $\pm$ 2.1), slightly lower than those in dentine of 26-42 days (Mean $\pm$ SD=35 $\pm$ 1.4). From the measurements we found that a constant 100 $\mu$ m ablation pit represented on a maximum of 42 days (6 weeks) of dentine growth. It seems this pit size minimises element fractions during LA-ICP MS analyses from previous study. [323] Accordingly, a series of age ranges of 6 weeks duration were constructed allowing the LA-ICP-MS elemental data to be assigned a chronological age. Lead ratios from each ablation pit were assigned to one of these age ranges, which were then numbered from interval 1 to interval 10 in enamel and interval 1 to interval 32 in primary dentine. A chart of deciduous tooth development was used to clarify the point of each ablation pit (Figure 3.13) and the conversions of intervals to ages used in this study are given in Table 3.3.



**Figure 3.13** A chart of deciduous tooth development (Courtesy of Wendy Dirks)

**Table 3.3** Conversion of age intervals into age in days for enamel and dentine

Interval	Age	Interval	Age
Interval 1	-126 to -168 days	Interval 17	514-556 days
Interval 2	-84 to -126 days	Interval 18	556-598 days
Interval 3	-42 to -84 days	Interval 19	598-640 days
Interval 4	birth to -42 days	Interval 20	640-682 days
Interval 5	birth to 42 days	Interval 21	682-724 days
Interval 6	42-84 days	Interval 22	724-766 days
Interval 7	84-126 days	Interval 23	766-808 days
Interval 8	126-168 days	Interval 24	808-850 days
Interval 9	168-220 days	Interval 25	850-892 days
Interval 10	220-262 days	Interval 26	892-934 days
Interval 11	262-304 days	Interval 27	934-976 days
Interval 12	304-346 days	Interval 28	976-1018 days
Interval 13	346-388 days	Interval 29	1018-1060 days
Interval 14	388-430 days	Interval 30	1060-1102 days
Interval 15	430-472 days	Interval 31	1102-1144 days
Interval 16	472-514 days	Interval 32	1144-1186 days

### 3.3.9 Analytical strategy

Given the small number of data points by age interval, formal statistical analysis of consistency was not able to be carried out. Lead ratios were plotted by age interval to enable a visual inspection of within and between tooth variations in lead ratio by age. Mean and standard deviations were used to represent the daily secretion rates of enamel and dentine and the lead ratios across the tooth surface. The consistent lead ratios at the same age were observed graphically as shown throughout my thesis. To test whether lead ratios were consistent at the same age across the coronal dentine, the ratios were plotted against age interval and compared in the two teeth from the same child. Means and standard deviations were used to represent the daily secretion rates of enamel and dentine and the lead ratios across the tooth surface. For the initial four teeth, scatter charts were used to assess intra-individual variability among transects of each tooth. Two sigma standard errors (2 Sigma S.E.) for each point were identified, where the 2 sigma S.E. is the sum of the errors for lead and calcium concentrations. In the LA-ICP-MS analysis, data processing provided the error summary ( $\mu\text{g/g}$ ) for each ablation point. The percentage of error for each point was calculated using the error summary ( $\mu\text{g/g}$ ) divided by the lead concentrations ( $\mu\text{g/g}$ ). Multiplied by 100, the results are then called the 1

sigma error (1 sigma S.E.) for the lead ratio, which is multiplied by 2 to produce the 2 sigma standard errors for every ablation point.

Once it was established that each age interval showed consistent lead levels no matter where it was sampled across the coronal dentine, the method was modified for the remaining teeth. One ablation transect was then applied to the remaining teeth in order to examine the history of exposure, as detailed in the next section. Owing to the rapidly increasing lead ratios found on approaching the pulp cavity, polarised-light microscopy was used to identify the different areas of the dentine (primary, secondary and tertiary dentine).

### **3.4 Phase III: Application of the histological technique to remaining teeth sample**

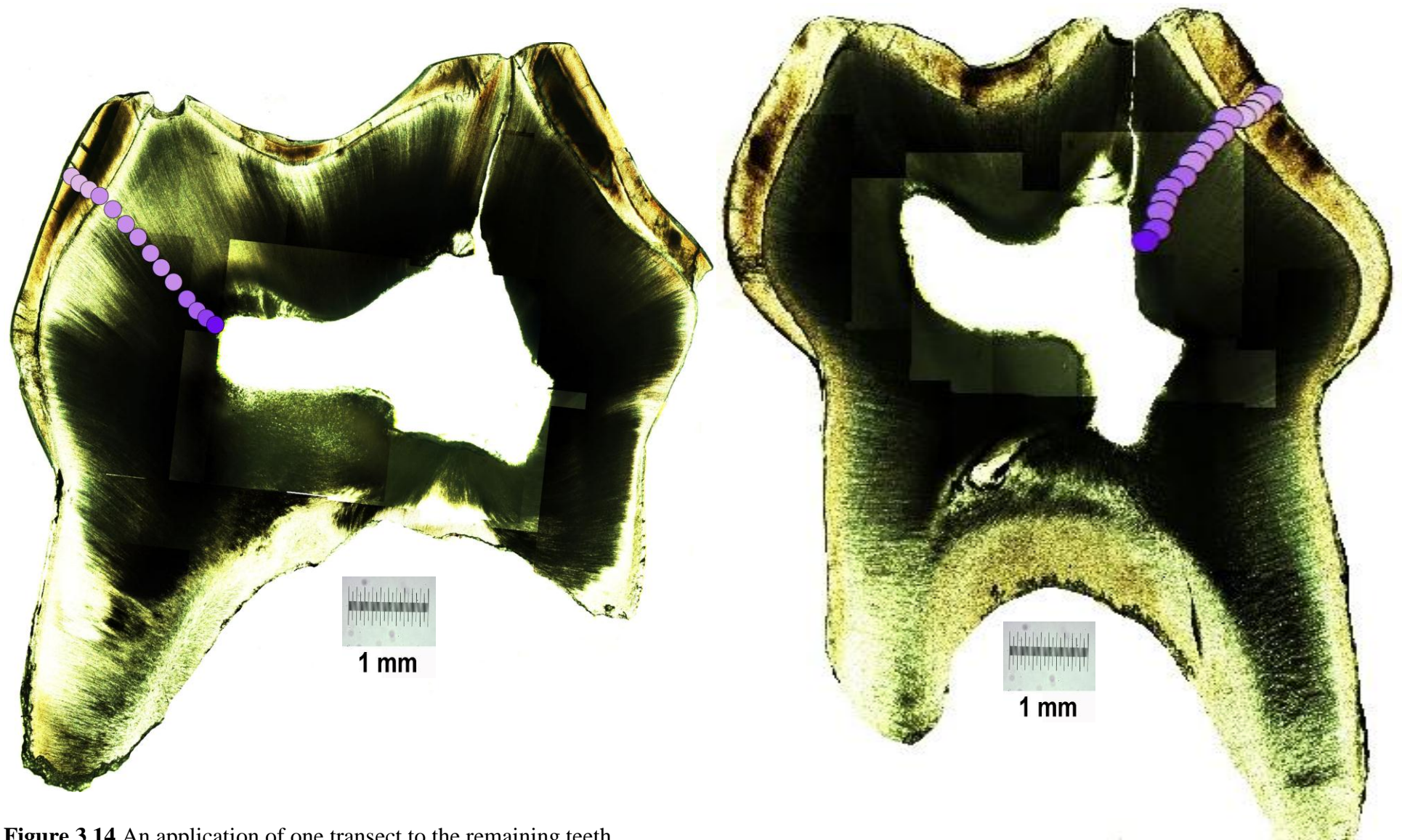
Based on the findings from the phase II, the lead ratios found were consistent at the same age interval in primary dentine, and increased sharply in the dentine near the pulp cavity. One ablation transect was therefore sampled in the 15 remaining teeth in order to determine the history of lead exposure.

Cross striations in the enamel for these 15 teeth were measured and found to range from 3.04-3.97 $\mu$ m (mean $\pm$ SD=3.34 $\pm$ 0.21), which is slightly less than those in the initial four teeth. In dentine, the von Ebner lines were measured at between 2.75  $\mu$ m and 3.01  $\mu$ m (mean $\pm$ SD=2.91 $\pm$ 0.05), which is almost the same as found in the initial four teeth.

The histological technique detailed above was used to identify the age of each ablation pit in the 15 remaining teeth. The number of days within ablation pits in enamel ranged from 27-38 days (Mean $\pm$ SD=30.7 $\pm$ 1.5) which is slightly lower than those in dentine of 29-39 days (Mean $\pm$ SD=33.8 $\pm$ 0.9). The same series of age ranges of six weeks (42 days) were used to encompass the maximum number of days within an ablation pit, and the lead ratio from each ablation pit was assigned to one of these age ranges. Figure 3.14 shows an application of one ablation transect to one pair from the remaining teeth.

Although the blood lead levels of 15 children were recorded, the BLLs of only 11 children were used to assess the relationship between tooth and blood lead levels. Four

pairs (4/15) were not be able to analyse tooth lead levels due to caries. Spearman's rank test was used to assess the relationship between BLLs and tooth lead levels and the children's age.



**Figure 3.14** An application of one transect to the remaining teeth



# **CHAPTER FOUR**

## **RESULTS**

# CHAPTER FOUR

## RESULTS

In the results chapter I will first present the characteristics of the participants of the two study cohorts used in my research, the Tooth Fairy Study and the Teesside samples. I will then present the results of the three phases of my study to address the key questions of my research:

1. **Are mean dentine lead levels associated with known determinants of exposure?** To address this question I use the findings from phase I: the Tooth Fairy Study.
2. **Can our histological technique recreate the history of lead exposure over a child's life time?** To address this question I present the key findings from Phases II and III, the Teesside samples. I will present the results through a process of asking four further questions.  
**Question 2a: Should we sample enamel and/or dentine?**  
**Question 2b: How many transects per tooth are required?**  
**Question 2c: How many teeth per child are required?**  
**Question 2d: Does tooth type matter?**
3. **What is the relationship between tooth and blood lead levels?** To address this question I will present findings from Phase II and III of my study.

### 4.1 Characteristics of the study participants

#### 4.1.1. Tooth Fairy Study

##### **A: Participants and their main place of residence**

The characteristics of the children and their places of residence when sampled are given in Table 4.1. Sixty-nine children (36 female, 33 male) were recruited, between the ages of 5 and 8 years old, with a modal age of 6 years.



Children were recruited from seventeen schools located within the Newcastle city boundaries (Figure 3.1). Sixty four percent of the children attended 5 local schools (Cragside Primary School 24.6%, Archbishop Runcie Church of England First School (14.5%), St Oswald's Catholic Primary School (10.1%), and Grange First School as well as Waverly Primary School (7.3%). The remaining 36% studied at 12 other local schools (see Figure 3.1).

Nearly 6% of the children had lived in different addresses in their earlier life; however, all children lived in Newcastle when recruited into the study. 67% of children had lived in their current place of residence for at least 5 years. With regards to the number of people living in the household, the majority of children (n=61, 88.4%) lived with two adults, 51.5% lived with at least two children aged 5 and over, and 84.6% lived with one child aged under 5 in the same house.

Around 44% of the children lived in homes built between 1931 and 1960. Most of the children (82.6%) lived in houses which had been renovated. About three-quarters of the children lived in houses where paintwork was in good condition with no flaking or peeling. For those house renovations, old paint (interior or exterior) being burnt off, sanded or water blasted was most often selected (43.5%) followed by the demolition of ceilings, floors and walls (37.7%) and stripping old paint with chemicals (10.1%).

**Table 4.1** Characteristics of children and their main place of residence

<b>Characteristics</b>	<b>n(n=69)</b>	<b>%</b>
<b>1. Sex</b>		
Male	33	47.8
Female	36	52.2
<b>2. Age (year)</b>		
5	9	13.0
6	32	46.4
7	25	36.2
8	3	4.3
<b>3. Schools (tertiles of IMD scores)</b>		
≤ 10.00 (least deprived)	2	2.9
10.01-20.00	34	49.3
20.01-30.00	24	34.8
≥ 30.01 (most deprived)	9	13.0
<b>4. When houses were built (year)</b>		
≤ 1930	19	27.9
1931-1960	30	44.2
≥ 1961	19	27.9
<b>5. IMD scores of place of residence (2007)</b>		
≤10 (least deprived)	25	36.2
10.01-20.00	12	17.4
20.01-30.00	8	11.6
≥30.01 (most deprived)	24	34.8
<b>6. Household income per month (2005)</b>		
417-1,249 GBP	15	21.7
1,250-2,083 GBP	21	30.4
2,084-2,916 GBP	13	18.8
≥2,917 GBP	20	29.0
<b>7. Home ever had lead water pipe</b>		
Yes	13	18.8
No	30	43.5
Not sure	26	37.7

<b>Characteristics</b>	<b>n(n=69)</b>	<b>%</b>
<b>8. House cleaning</b>		
<b>8.1 Frequency of house vacuuming</b>		
>1 time/week	44	63.8
Weekly	19	27.5
Less than weekly	6	8.7
<b>8.2 Frequency of cleaning hard floors in the house</b>		
>1 time/week	26	37.7
Weekly	10	14.5
Less than weekly	7	10.1
No hard floors in the houses	26	37.7
<b>8.3 Methods of cleaning hard floors in the house</b>		
Dry cleaned with a dust pan and brush or broom	2	4.7
Vacuum / Wet cleaned with mop	27	62.8
Both methods above	14	32.6
<b>9. Number of people living in house</b>		
<b>9.1 Number of adults aged over 16 years</b>		
1	8	11.6
≥ 2	61	88.4
<b>9.2 Number of children aged 5 and over</b>		
1	24	35.3
2	35	51.5
≥ 3	9	13.2

## **B: Characteristics of child's eating and drinking behaviour and health**

A total of 17 (24.6%) of the children lived in houses where fruit and vegetables were grown in an allotment plot or in the garden, and of these 13 (76.5%) ate the fruits and vegetables grown there. Just over half (55.1%) of the children drank between ½ pint and 1 pint of tap water per day, 24.6% of the children drank more than one pint, and 20.3% drank less than ½ pint daily. The majority of the children (84.1%) had received bottle milk feeds (not including expressed milk) but only 19 children (27.5%) had received bottle feeds within 1 month from birth, the remaining 39 children (57%) started bottle feeds more than 1 month from birth.

Parents were asked about their child's hand-to-mouth behaviour that could possibly be related to lead contamination. 6 children (8.7%) sucked their thumbs. Only one child chewed or sucked painted objects, and one child put small toys or other objects such as buttons into his mouth. One child was identified who ate soil, dirt and possibly creatures living in soil such as earthworms. Only one parent reported that food was stored in pottery or ceramics with leaded glazed or lead crystal/pewter. None of the children ate food stored in lead soldered cans.

The majority of the children (94.2%) had no brother or sister with elevated blood lead levels: the remaining 5.8% were unsure about their siblings blood lead levels. One child or member of their household reported the use of traditional Asian cosmetics.

Information about medical conditions was obtained. Only 9 children (13%) suffered from medical conditions such as asthma and eczema. One child had been diagnosed with calcium or iron deficiency (anaemia), and this child had also lived with a household member involved in electronics and car repair. 8 children (11.6%) had ever taken alternative or traditional remedies.

## **C: Socio-economic status and occupational characteristics**

Parents were asked about their highest educational levels and monthly income. A total of 41 (59.5%) of the main wage earners in households were well educated, having attended polytechnic or university, 13 (18.8%) had attended colleges of further education or other types of college, 15 (21.7%) had attended only primary and secondary school.

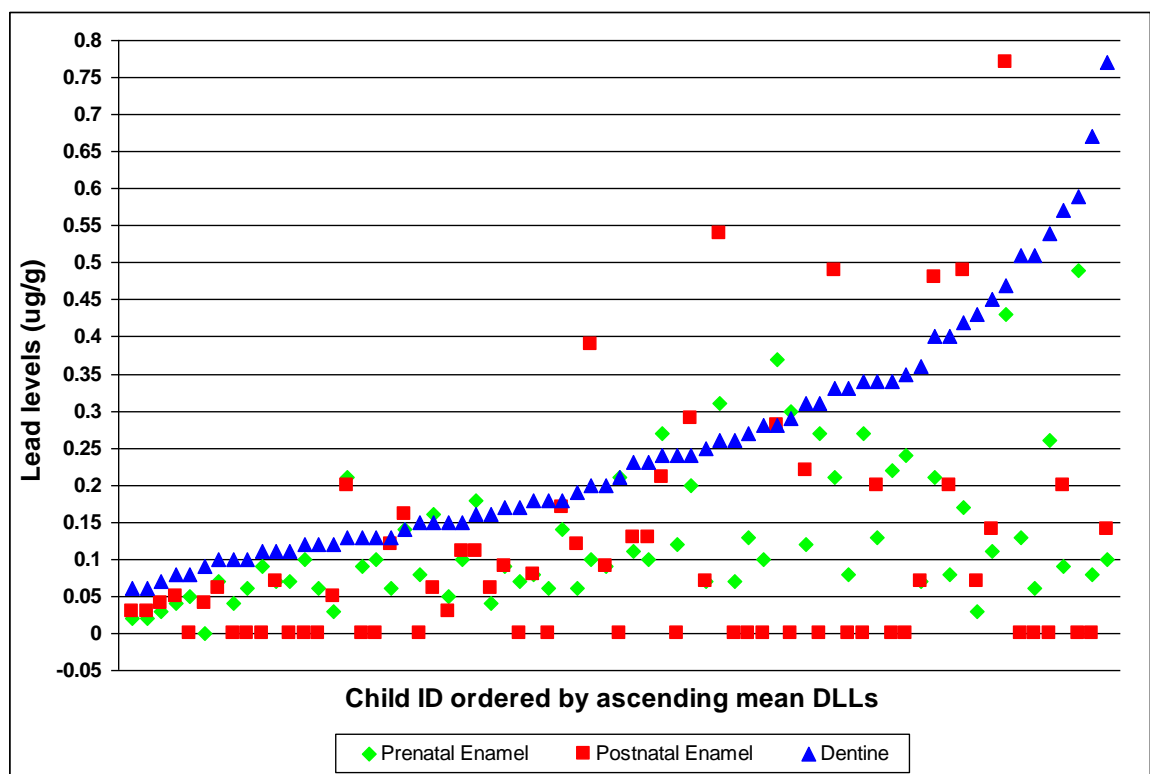
Parents were asked about specific occupations that might result in exposure and one third of the parents participated in these activities. Of these, electronics (15.9%), followed by car repair (14.5%), floor sanding (7.2%), and car battery service/repair or manufacture (4.3%) were reported. One parent reported that a household member was involved in electronics as well as car repair and floor sanding. Three indicated that household members did electronic activity as well as car repair.

It was found that 13 of the children (18.8%) lived in the houses where household members had smoked.

IMD scores were used to classify the level of deprivation of the area of each child's place of residence and school. For living areas, the 25<sup>th</sup> percentile was equal to an IMD score of 10. I then divided IMD scores into four categories as follows:  $\leq 10$ , 10.01-20.00, 20.01-30.00 and  $\geq 30.01$ . Just over half (53.6%) of the children lived in the least deprived areas (IMD score  $\leq 10$ ). Regarding IMD Scores of schools, I also classified these into the same 4 categories as the IMD of residence. Only 2 children attended schools located in the least deprived areas. Most of the children (~84%) attended schools moderately deprived areas (IMD scores of 10.01-30.00), and the remaining 13% attended schools located in the most deprived areas (IMD  $\geq 30.01$ ).

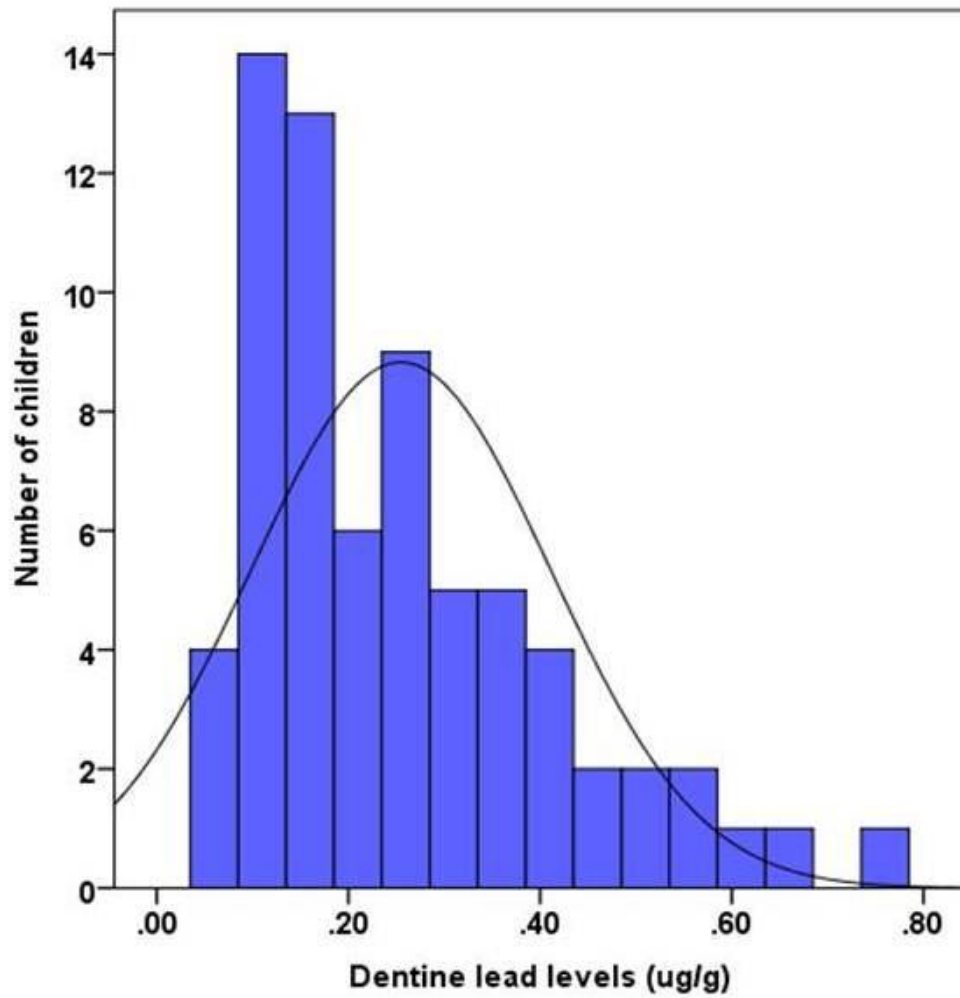
## D: Dentine lead levels in children from the Tooth Fairy Study

The Tooth Fairy study involved the collection of deciduous teeth and analysis of lead levels in prenatal and postnatal enamel and dentine. Mean lead levels in pre- and postnatal enamel were lower overall than DLLs, and tended to be lower in each, although not every child, as shown in Figure 4.1. Although 68/69 children had detectable lead levels in prenatal enamel, only 41/69 children had detectable lead levels in their postnatal enamel, making enamel a less useful matrix for exploring lead in low exposure populations. All children had detectable levels of lead in dentine, and because dentine captures a potentially greater period of exposure history, DLLs were chosen as the potential biomarker to study determinants of lead exposure.



**Figure 4.1** Mean enamel and dentine lead levels in children recruited in the Tooth Fairy Study

DLLs ranged between 0.06-0.77ug/g, (Mean $\pm$ SD=0.25 $\pm$ 0.16). Figure 4.2 shows the distribution of mean dentine lead levels in the Tooth Fairy study, indicating that these data are positively skewed.



**Figure 4.2** The histogram of mean DLLs in children in the Tooth Fairy Study

## 4.1.2 Details of participants recruited into the Teesside samples

Fifteen children from Teesside were recruited for this study and two extracted teeth were collected from each child. Eight teeth from 4 participants (HT:4,7,10,15) and one tooth for child HT6 (tooth 65) could not be used for laser ablation due to caries and attrition. Of the 21 remaining teeth, one tooth from the child with the highest BLL (HT6-74) and another tooth (HT8-84) were ablated but subsequently excluded from the histological study due to difficulties identifying the neonatal line in dentine. Details of age, sex, tooth type and blood lead levels of the fifteen children sampled are given in Table 4.2. Of the 15 children, 9 (60%) were male, and 6 (40%) were female. The ages of these children ranged from 6-8 years old, with mean 6.9.

**Table 4.2** Details of fifteen Teesside children recruited in the histological study

No.	Children ID	Sex	Tooth types		Age (years)	BLLs (µg/dL)
			Tooth 1	Tooth 2		
1	HT1	Female	55	54	6	1.01
2	HT2	Male	54	64	7	1.14
3	HT3	Female	55	65	7	2.18
4	HT4	Male	+	+	6	1.47
5	HT5	Female	65	54	8	1.33
6	HT6	Female	+	74*	8	6.83
7	HT7	Male	+	+	6	0.80
8	HT8	Male	84*	85	7	0.76
9	HT9	Female	55	65	6	1.45
10	HT10	Male	+	+	8	1.22
11	HT11	Male	85	64	6	0.52
12	HT12	Male	85	84	8	0.93
13	HT13	Female	75	84	8	1.36
14	HT14	Male	74	84	7	0.71
15	HT15	Male	+	+	6	1.32
<b>Mean (SD)</b>					<b>6.9 (0.9)</b>	<b>1.53 (1.5)</b>
<b>Median</b>					<b>7</b>	<b>1.22</b>

+ Unable to be used

\* Excluded from histological study



## 4.2 Question 1: Are mean DLLs associated with known determinants of lead exposure?

### 4.2.1 Univariate analyses

Results of the univariate analyses to identify the determinants of DLLs are shown in Table 4.3. Mean DLLs were slightly lower in males ( $0.25\mu\text{g/g}$ ) than in females ( $0.26\mu\text{g/g}$ ), but this difference was not statistically significant ( $p>0.05$ ). DLLs were higher in children  $\geq 7$  years old ( $0.28\mu\text{g/g}$ ) than those younger children ( $\leq 0.26\mu\text{g/g}$ ), but again the difference was not statistically significant. There was no significant difference between DLLs in children from different schools. Children attending Waverly Primary school had the highest DLLs ( $0.36\mu\text{g/g}$ ), followed by Grange First school ( $0.32\mu\text{g/g}$ ) and Cragside Primary school ( $0.29\mu\text{g/g}$ ), and the mean lead levels in dentine from those attending the remaining schools ranged from  $0.18$ - $0.24\mu\text{g/g}$ . When classifying schools into four categories using the IMD score of the local area, children who studied in schools in moderately deprived areas (IMD scores  $20.01$ - $30.00$ ) had the highest mean DLL ( $0.28\mu\text{g/g}$ ), followed by lesser deprived IMD scores  $10.01$ - $20.00$  ( $0.25\mu\text{g/g}$ ) and most deprived IMD scores  $\geq 30.01$  ( $0.23\mu\text{g/g}$ ). While few children attended schools in the least deprived areas (IMD scores  $\leq 10.00$ ) had mean DLLs  $0.17\mu\text{g/g}$ . However, no statistically significant association between DLLs and the IMD scores of schools was found.

Children from houses with lead water pipes had higher DLLs ( $0.30\mu\text{g/g}$ ) than those without ( $0.24\mu\text{g/g}$ ) or those who were not sure about the type of water pipes ( $0.25\mu\text{g/g}$ ), but this difference was not significant. Children living in houses built before 1960 had higher DLLs ( $0.27\mu\text{g/g}$ ) than those living in houses built after 1960 ( $0.23\mu\text{g/g}$ ), but no statistical differences in DLLs was found between these two groups.

In terms of the frequency of house vacuuming, having hard floors in the house, and the frequency of cleaning hard floors, no associations were found with DLLs in children. The mean DLL was significantly higher in children whose houses were cleaned using only a dust pan and brush or broom ( $0.67\mu\text{g/g}$ ) compared to those whose houses were cleaned using vacuum/wet cleaned with mop (mean= $0.22\mu\text{g/g}$ ,  $p=0.01$ ), however, only 2 children populated this former group. Children living in houses where hard floors were cleaned

less than weekly had higher dentine lead levels ( $0.34\mu\text{g/g}$ ) than those living in houses where floors were cleaned weekly or more often ( $\leq 0.28\mu\text{g/g}$ ), but this difference was not statistically significant.

There was no statistically significant difference between DLLs in children living in houses with paintwork in good condition and those with some flaking and/or peeling paint. With respect to renovations, those reporting renovation had DLLs not significantly different from those living in houses without any such renovation.

Children from households without home-grown fruit and vegetables had higher DLLs ( $0.28\mu\text{g/g}$ ) than those growing fruits/vegetables ( $0.19\mu\text{g/g}$ ), and this difference was statistically significant ( $p < 0.05$ ). However, no statistically significant difference was found between DLLs in children who ate those fruits/vegetables and those who did not.

Drinking behaviour and the amount of tap water that the child drank daily was found to be a significant determinant of dentine lead levels ( $p < 0.05$ ). Children who drank less than 1/2 pint tap water per day had higher DLLs ( $0.34\mu\text{g/g}$ ) than those who reportedly drank more ( $0.22\text{--}0.26\mu\text{g/g}$ ). Children who had been bottle fed milk had slightly higher DLLs ( $0.26\mu\text{g/g}$ ) than those who had not ( $0.23\mu\text{g/g}$ ), but this difference was not statistically significant. No statistically significant difference was found between DLLs in children starting bottle feeding at  $< 1$  versus  $> 1$  month from birth.

With respect to hand-to-mouth behaviours, children who currently or had ever sucked their thumbs had slightly higher DLLs ( $0.27\mu\text{g/g}$ ) than those who did not ( $0.25\mu\text{g/g}$ ), but this difference was not statistically significant. The child who reportedly chewed or sucked painted objects or put small toys in their mouth had a higher DLL ( $0.30\mu\text{g/g}$ ) but this difference was not statistically significant.

Reported medical conditions were not statistically significantly associated with higher DLLs. Eczema was most often found among these children ( $n=5$ ), with asthma ( $n=3$ ), and chest infection ( $n=1$ ) also reported. Children who suffered from any of these medical conditions had higher mean DLLs ( $0.31\mu\text{g/g}$ ) than those who did not ( $0.25\mu\text{g/g}$ ), but the difference was not statistically significant. Children who had ever been given traditional

remedies had slightly higher DLLs ( $0.26\mu\text{g/g}$ ) than those who had never had those remedies ( $0.25\mu\text{g/g}$ ), but this difference was not statistically significant.

With respect to measures of socio-economic status, there was not statistically significant effect of educational level of the main wage earner in the household on DLLs in children. Children from households with more highly educated members had comparable dentine lead levels ( $0.26\mu\text{g/g}$ ) to those where parents had attended only primary and secondary schools ( $0.26\mu\text{g/g}$ ). DLLs were lower in households where the main wage earners attended colleges of further education ( $0.23\mu\text{g/g}$ ), but this difference was not statistically significant.

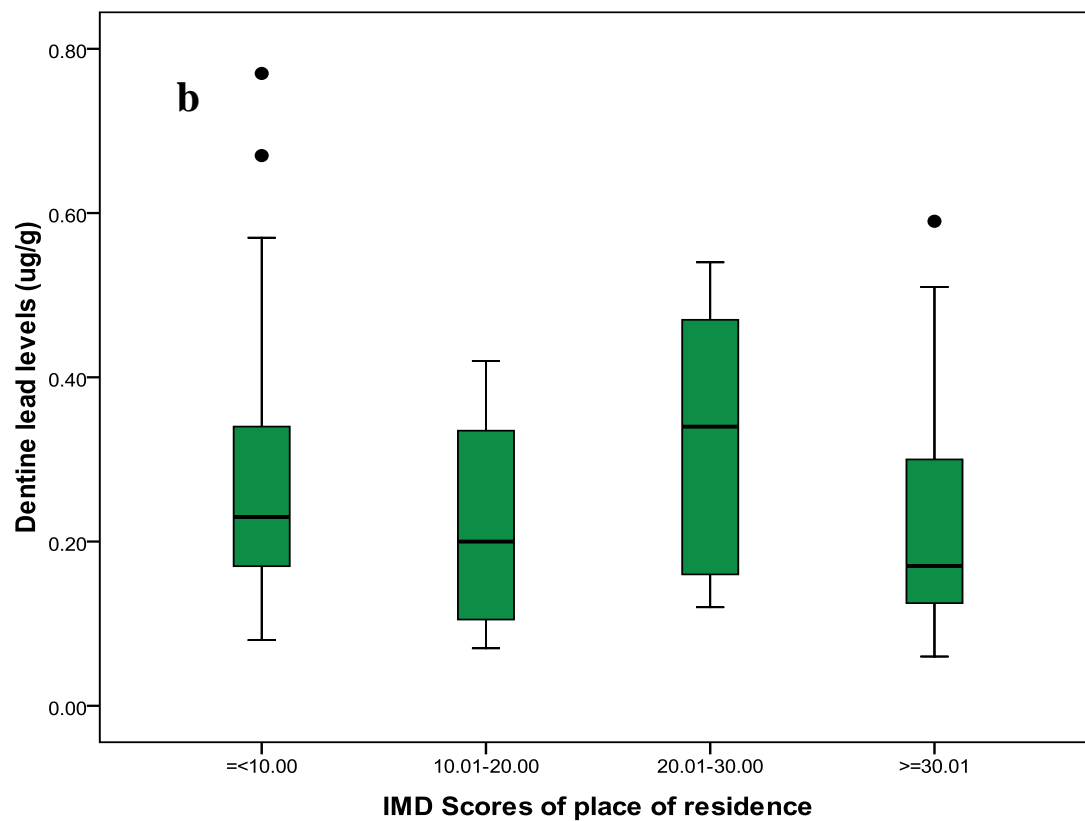
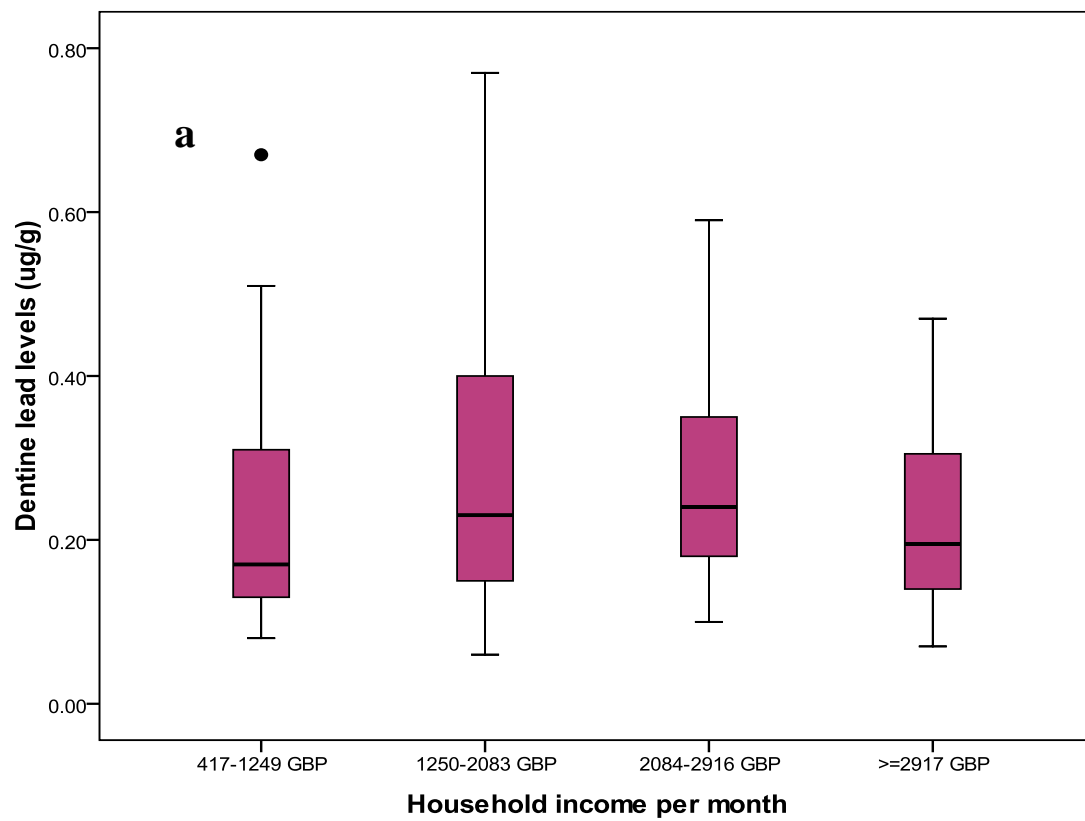
No statistically significant effect on DLLs was found for household income. The highest DLLs ( $0.28\mu\text{g/g}$ ) were found in children from households earning middle incomes (1,250 to 2,916 GBP monthly). Children with the lowest household income (417-1,249 GBP monthly) had the lowest DLLs ( $0.22\mu\text{g/g}$ ). Figure 4.3a shows DLLs in children from different household income groups.

Regarding IMD scores of their place of residence, children living in moderately deprived areas (IMD scores 20.01-30.00) had the highest DLLs ( $0.33\mu\text{g/g}$ ) compared to those living in the least deprived areas ( $0.28\mu\text{g/g}$ ) and most deprived areas ( $0.22\mu\text{g/g}$ ). However, these differences were not statistically significant (see Figure 4.3b). DLLs by child's postcode are superimposed over IMD scores by LSOA is shown in Figure 4.4.

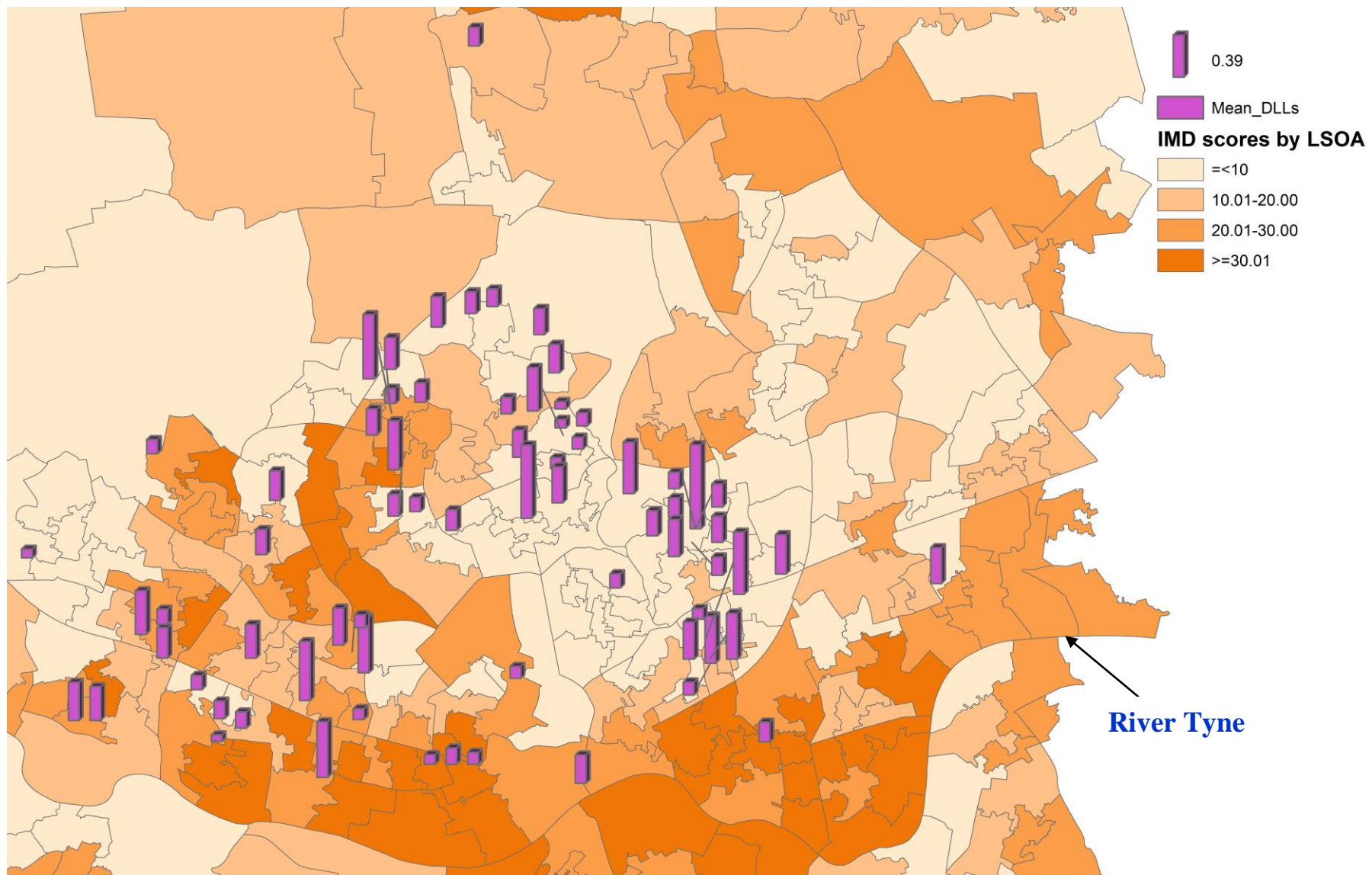
There was no statistically significant relationship between DLLs and the number of people living in the main residence. Children who had only one adult present had higher DLLs ( $0.31\mu\text{g/g}$ ) than those who had two or more adults in the house ( $0.25\mu\text{g/g}$ ), but this difference was not statistically significant. No difference was found between DLLs and number of children aged 5 and over living in the same home.

A higher mean DLL was found in children whose household members had smoked ( $0.27\mu\text{g/g}$ ) compared to those who did not ( $0.25\mu\text{g/g}$ ), but this difference was not statistically significant.

Regarding children's contact with household members whose jobs or activities involved car repair, panel-beating, lead-lighting, lead window-making, lead weight making, electronics, ammunition handling, car-battery servicing or manufacture and floor-sanding, these activities were not shown to influence the DLLs of the children in this study, although only a few cases were affected.



**Figure 4.3** DLLs by a. household income and b. IMD scores of place of residence



**Figure 4.4** Map showing DLLs in children living in Newcastle divided by IMD scores by LSOA

**Table 4.3** Associations between child household characteristics and DLLs

<b>Factors</b>	<b>n</b>	<b>%</b>	<b>Mean</b>	<b>SD</b>	<b>p-value</b>
<b>1. Sex</b>	<b>69</b>				<b>0.80</b>
Male	33	47.8	0.25	0.13	
Female	36	52.2	0.26	0.17	
<b>2. Age (years)</b>	<b>69</b>				<b>0.60</b>
5	9	13.0	0.26	0.13	
6	32	46.4	0.24	0.17	
≥7	28	40.6	0.28	0.15	
<b>3. Schools (by IMD scores)</b>	<b>69</b>				<b>0.71</b>
≤10.00 (least deprived)	2	2.9	0.17	0.12	
10.01-20.00	34	49.3	0.25	0.15	
20.01-30.00	24	34.8	0.28	0.16	
≥30.01 (most deprived)	9	13.0	0.23	0.18	
<b>4. Home ever had lead water pipe</b>	<b>69</b>				<b>0.59</b>
Yes	13	18.8	0.30	0.19	
No	30	43.5	0.24	0.15	
Not sure	26	37.7	0.25	0.16	
<b>5. Years that house was built</b>	<b>68</b>				<b>0.60</b>
≤1930	19	27.9	0.26	0.13	
1931-1960	30	44.2	0.27	0.18	
≥1961	19	27.9	0.23	0.14	
<b>6. House cleaning</b>					
<b>6.1 Frequency of house vacuuming</b>	<b>69</b>				<b>0.44</b>
>1 time/wk	44	63.8	0.24	0.15	
Weekly	19	27.5	0.29	0.18	
Less than weekly	6	8.7	0.22	0.13	
<b>6.2. Hard floor in house</b>	<b>69</b>				<b>0.33</b>
Yes	43	62.3	0.27	0.17	
No	26	37.7	0.23	0.12	

<b>Factors</b>	<b>n</b>	<b>%</b>	<b>Mean</b>	<b>SD</b>	<b>p-value</b>
<b>6.3 Frequency of cleaning hard floor in the house</b>	<b>43</b>				<b>0.14</b>
>1 time/wk	26	60.5	0.28	0.18	
Weekly	10	23.3	0.18	0.07	
Less than weekly	7	16.2	0.34	0.22	
<b>6.4 Methods of cleaning hard floors in the house</b>	<b>43</b>				<b>0.01**</b>
Dry cleaned with a dust pan and brush or broom	2	4.7	0.67	0.14	
Vacuum / Wet cleaned with mop	27	62.8	0.22	0.14	
Both methods above	14	32.5	0.30	0.15	
<b>7. General condition of the interior paintwork</b>	<b>69</b>				<b>0.80</b>
Paint is in good condition with no flaking or peeling	51	73.9	0.25	0.15	
There is some flaking or peeling	18	26.1	0.26	0.17	
<b>8. Renovation</b>	<b>69</b>				<b>0.49</b>
Yes	57	82.6	0.26	0.16	
No	12	17.4	0.23	0.15	
<b>8.1 Old paint sanded or water blasted</b>	<b>69</b>				<b>0.30</b>
Yes	30	43.5	0.28	0.17	
No	39	56.5	0.24	0.14	
<b>8.2 Old paint stripped with chemical</b>	<b>69</b>				<b>0.22</b>
Yes	7	10.1	0.32	0.25	
No	62	89.9	0.25	0.14	
<b>8.3 Ceiling, floor or wall demolished</b>	<b>69</b>				<b>0.91</b>
Yes	26	37.7	0.26	0.17	
No	43	62.3	0.25	0.15	
<b>8.4 None of the renovations above</b>	<b>69</b>				<b>0.86</b>
Yes	17	24.6	0.26	0.15	
No	52	75.4	0.25	0.16	
<b>9. Growing fruits or vegetable in the house's garden</b>	<b>69</b>				<b>0.05**</b>
Yes	17	24.6	0.19	0.10	
No	52	75.4	0.28	0.17	

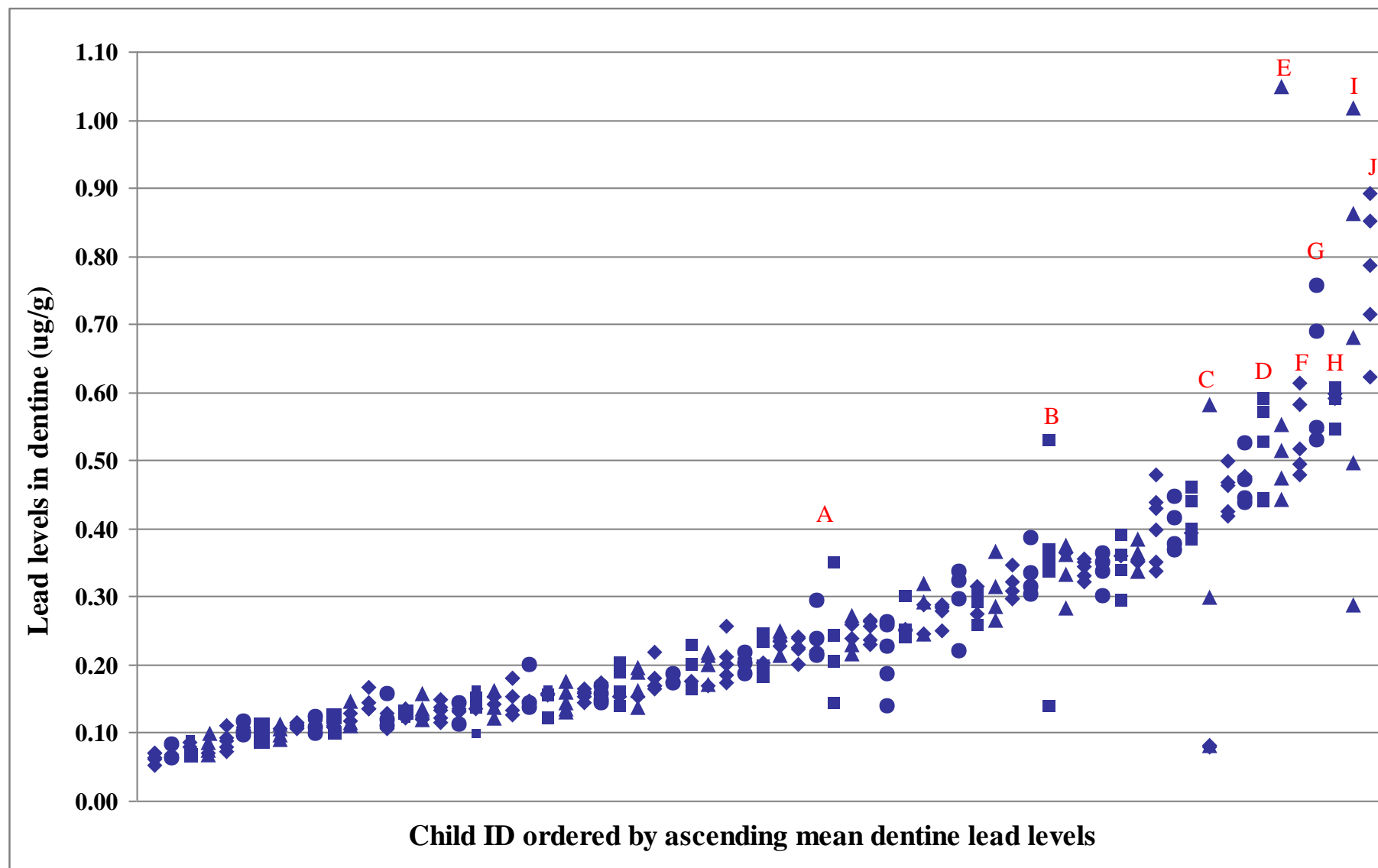


<b>Factors</b>	<b>n</b>	<b>%</b>	<b>Mean</b>	<b>SD</b>	<b>p-value</b>
<b>9.1 Eating fruits or vegetable grown in the house's garden</b>	<b>17</b>				<b>0.77</b>
Yes	13	76.5	0.19	0.11	
No	4	23.5	0.21	0.10	
<b>10. Amount of tap water that child drinks daily</b>	<b>69</b>				<b>0.04**</b>
Up to 1/2 pint	14	20.3	0.34	0.17	
1/2 pint to 1 pint	38	55.1	0.22	0.15	
More than one pint	17	24.6	0.26	0.13	
<b>10.1 Child ever received bottle feeds</b>	<b>69</b>				<b>0.51</b>
Yes	58	84.1	0.26	0.17	
No	11	15.9	0.23	0.09	
<b>10.2 Child received bottle feeds at age:</b>	<b>69</b>				<b>0.36</b>
Less than 1 month	19	27.5	0.27	0.18	
1-12 months	19	27.5	0.28	0.18	
≥13 months	20	29.0	0.23	0.14	
Never	11	16.0	0.23	0.15	
<b>11. Hand to mouth behaviours</b>					
<b>11.1 Child sucks his thumb</b>	<b>69</b>				<b>0.84</b>
Yes and ever sucked but not currently	6	8.7	0.27	0.14	
No and never has	63	91.3	0.25	0.16	
<b>11.2 Child chews/sucks painted objects/has small toy in mouth</b>	<b>69</b>				<b>0.30</b>
Yes and ever sucks but not currently	6	8.7	0.30	0.14	
No and never has	63	91.3	0.25	0.16	
<b>12. Medical conditions</b>					
<b>12.1 Child suffered from medical conditions</b>	<b>69</b>				<b>0.31</b>
Yes	9	13.0	0.31	0.17	
No	60	87.0	0.25	0.15	
<b>12.2 Child has traditional remedies</b>	<b>69</b>				<b>0.91</b>
Yes	8	11.6	0.26	0.17	
No	61	88.4	0.25	0.16	

<b>Factors</b>	<b>n</b>	<b>%</b>	<b>Mean</b>	<b>SD</b>	<b>p-value</b>
<b>13. Socio-economic status</b>					
<b>13.1 Highest education of the main wage earner in the household</b>	<b>69</b>				<b>0.83</b>
Primary or Secondary school	15	21.7	0.26	0.17	
College of further education	13	18.8	0.23	0.17	
Polytechnic or University	41	59.5	0.26	0.15	
<b>13.2 Household income per month</b>	<b>69</b>				<b>0.64</b>
417-1,249 GBP	15	21.7	0.22	0.17	
1,250-2,083 GBP	21	30.4	0.28	0.19	
2,084-2,916 GBP	13	18.8	0.28	0.15	
≥2,917 GBP	20	29.0	0.24	0.11	
<b>13.3 IMD scores of place of residence (2007)</b>	<b>69</b>				<b>0.26</b>
≤10 (least deprived)	25	36.2	0.28	0.18	
10.01-20.00	12	17.4	0.23	0.13	
20.01-30.00	8	11.6	0.33	0.17	
≥30.01(most deprived)	24	34.8	0.22	0.12	
<b>14. Number of people living in the house</b>					
<b>14.1 Number of adults aged over 16 years</b>	<b>69</b>				<b>0.28</b>
1	8	11.6	0.31	0.16	
≥ 2	61	88.4	0.25	0.16	
<b>14.2 Number of children aged 5 and over</b>	<b>68</b>				<b>0.46</b>
1	24	35.3	0.22	0.13	
2	35	51.5	0.27	0.17	
≥ 3	9	13.2	0.27	0.17	
<b>15. Household members smoked in the main place of residence</b>	<b>69</b>				<b>0.65</b>
Yes	13	18.8	0.27	0.18	
No	54	78.2	0.25	0.15	
<b>16. Household members have taken part in activities with potential lead exposure</b>					
<b>16.1 Car repair</b>	<b>69</b>				<b>0.90</b>
Yes	10	14.5	0.26	0.19	
No	59	85.5	0.25	0.15	

<b>Factors</b>	<b>n</b>	<b>%</b>	<b>Mean</b>	<b>SD</b>	<b>p-value</b>
<b>16.2 Electronics</b>	<b>69</b>				<b>0.72</b>
Yes	11	15.9	0.27	0.17	
No	58	84.1	0.25	0.16	
<b>16.3 Car battery service/repair or manufacture</b>	<b>69</b>				<b>0.97</b>
Yes	3	4.3	0.26	0.24	
No	66	95.7	0.25	0.15	
<b>16.4 Floor sanding</b>	<b>69</b>				<b>0.55</b>
Yes	5	7.2	0.21	0.16	
No	64	92.8	0.26	0.16	
<b>16.5 None of the above activities</b>	<b>69</b>				<b>0.30</b>
Yes	43	62.3	0.27	0.16	
No	26	37.7	0.23	0.14	

Figure 4.5 shows the distribution of DLLs in each of the 4-6 ablation pits for each child. The majority of the children had mean DLLs < 0.5 µg/g (an arbitrary cut off). Seven children had DLLs > 0.5 µg/g (D-J). Of these 7 children, two (E and F) lived with a household member involved with electronics, and two (D and F) lived with a household member who worked in car repairs, and another child (G) had lived with parents who stored food in pottery or ceramic with lead glaze. Four of these children (E, G, I & J) had a statistically greater spread (SD > 0.08) of sampled DLLs than other children. Compared to a cut off mean DLL = 0.5 µg/g, these four children had higher median DLLs as for 0.54, 0.55, 0.68 and 0.78 µg/g respectively. A further three children (A, B and C) had DLLs less than 0.5 µg/g but showed a greater spread in DLLs across the sample pits.



**Figure 4.5** Distribution of DLLs across sampling points in each tooth from the Tooth Fairy study

## 4.2.2 Multivariate analyses

Multivariate linear regression was used to test the association between determinants of lead exposure and dentine lead levels, and the regression model is shown in Table 4.4.

Determinants entered into the regression model included sex, age, year that the house was built, household income, presence of lead water pipes in the house, frequency of cleaning hard floors in the house, growing fruits or vegetables in the gardens, and amount of tap water that the child drank daily. Four independent variables were eventually used in the final model: frequency of cleaning hard floors in the house, amount of tap water that the child drank daily, year that the house was built and household income per month as a categorised variable. However, none of these variables was significantly associated with DLLs.

**Table 4.4** Multiple regression analysis examining the determinants of DLLs

Variables	Coefficient	Std. Error	$\beta$	t	p-value
1. Frequency of cleaning hard floor in the house					
: > 1 time/week	0.13	0.06	0.38	2.08	0.46
: Less than weekly	0.20	0.08	0.43	2.46	0.19
: Weekly*	-				
2. Amount of tap water that child drank daily					
: Up to ½ pint	0.17	0.06	0.43	2.86	0.17
: More than one pint	0.03	0.06	0.07	0.45	0.66
: ½ pint to 1 pint*	-				
3. Year that house was built					
: ≤ 1930	-0.01	0.08	-0.03	-0.12	0.9
: 1931-1960	0.07	0.06	0.19	1.14	0.26
: ≥ 1961*	-				
4. Household income per month					
: 417-1,249 GBP	0.04	0.08	0.01	0.06	0.95
: 1,250-2,083 GBP	-0.01	0.07	-0.02	-0.09	0.93
: 2,084-2,916 GBP	0.14	0.09	0.28	1.59	0.12
: ≥2,917 GBP*	-				
<b>Constant</b> = 0.052 ; <b>Std. Error</b> = 0.077 ; <b>R</b> = 0.62 ; <b>R<sup>2</sup></b> = 0.38 ; <b>F</b> = 1.29 ; <b>p-value</b> = 0.42					

\*References category for variables in group

## Summary

**Question 1:** Are mean dentine lead levels associated with known determinants of exposure?

**Answer:** Unlike other studies, from the Tooth Fairy Study did not show a clear relationships between determinants of lead exposure and DLLs in children living in Newcastle.

The sampling pits in the Tooth Fairy study were located randomly as 4-6 points in the coronal dentine. Given the chronological development of dentine, these mean DLLs tell us something about lead exposure across various and inconsistently sampled ‘snapshots’ in time, meaning this biomarker potentially varies child by child, making interpretation difficult. We therefore decided that a more refined sampling strategy, using dental histology, was needed to develop an optimal and meaningful sampling technique. Earlier studies measured tooth lead levels and used the neonatal line to identify pre- and postnatal lead exposure. Humphrey and colleagues developed a novel sampling strategy for trace elements, including lead [245, 246, 253, 284, 324], using a combination of LA-ICP-MS and growth increments in enamel in histological tooth sections. Their work demonstrated that a detailed chronology of the incorporation of trace elements in enamel could be reconstructed using histological ground sections. Dentine also has incremental lines, and mineralises throughout the life of the tooth, and therefore held potential to record the detailed chronology of lead exposure. The second and third phases of my study aimed to reconstruct the life-time lead exposure in children by sampling dentine as well as enamel, modifying the techniques developed by Humphrey and colleagues.

## **4.3 Question 2: Can our histological technique recreate the history of lead exposure over a child's life time?**

### **4.3.1 Question 2a: Should we sample enamel and/or dentine?**

In the second phase of this study, two teeth from child HT1 (teeth 54 and 55) and two teeth from child HT11 (teeth 64 and 85) were selected for ablation to develop the histological technique. The criteria for choosing these two children were their sections were sound, and able to be sectioned in a way to reveal histology of the dentine. These two children had moderate and low BLLs, so would test if we could detect DLLs with our approach.

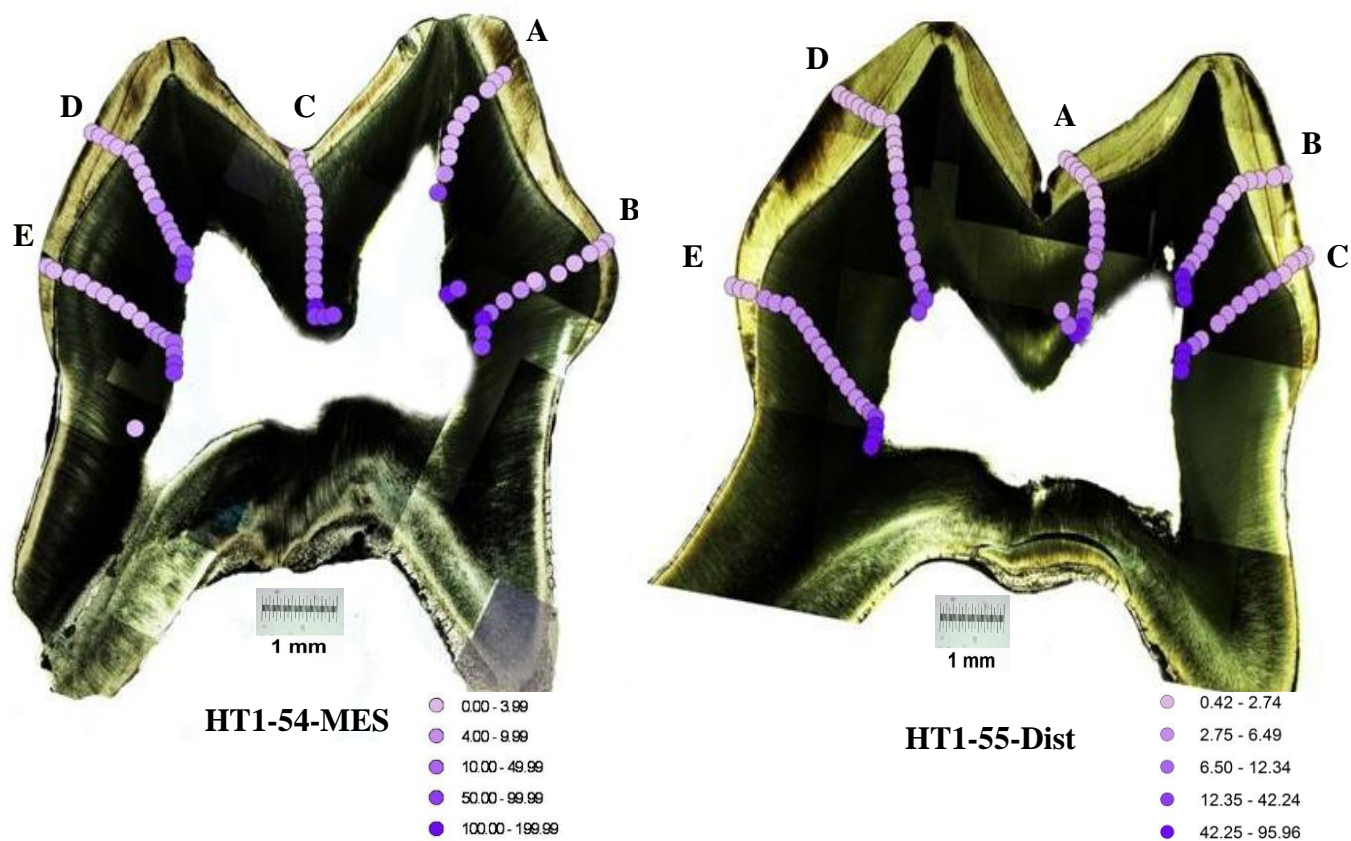
For each tooth surface, five transects (A-E) of around 2-7 ablation pits per transect in enamel and a minimum of 10 ablation pits per transect in dentine were sampled. The lead ratios were mapped using ArcGIS 9.3 software. Figure 4.6 shows the distribution of lead ratios across each tooth surface for the two teeth for child HT1 and Figure 4.7 shows the lead ratios by age interval across transects in dentine for this child.

For child HT1 (tooth 54), lead ratios were determined for five transects (A-E) at ablation points that included sampling points on either side of the neonatal line in both enamel and dentine. Lead ratio distributions in each transect showed a similar pattern, with comparatively low ratios of lead observed in enamel, while postnatally formed dentine showed a marked increase in lead ratio, particularly in the regions close to pulp cavity. Lead ratios slightly differed between pre- and postnatal enamel with ratios in pre- and postnatal enamel ranging from 0.44 to 1.37 (mean±SD=0.69±0.36) and 0.45 to 1.30 (mean±SD=0.8±0.31) respectively, but these ratios were not significantly different (there were only a small number of sampling points (1-5 points per transect)). In dentine, lead ratios in prenatal and postnatal dentine ranged from 2.5-3.21 (mean±SD=2.85±0.26) and 2.46-113.20 (mean±SD=14.15±26.53), respectively.

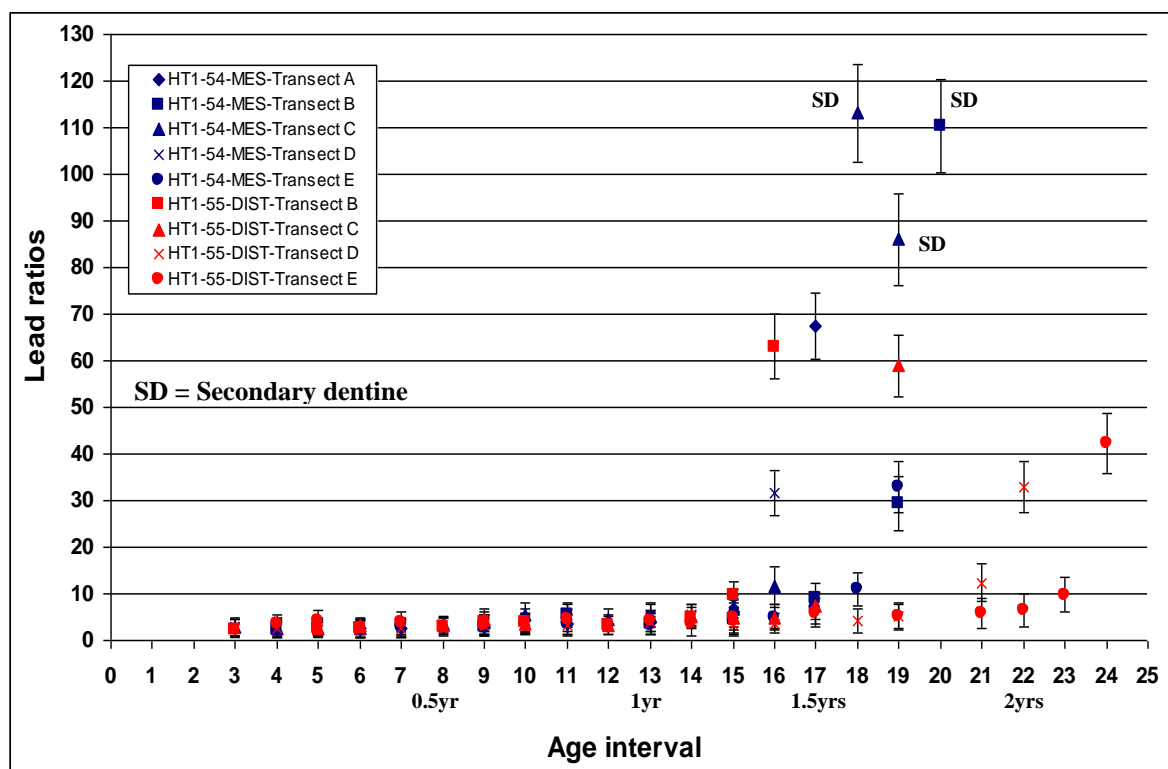
For tooth HT1-55, similar trends of lower lead levels in enamel and higher lead ratios in dentine were also found. Lead ratios in pre- and postnatal enamel ranging from 0.42 to 0.68 (mean±SD=0.57±0.1) and 0.46 to 2.15 (mean±SD=0.89±0.5), respectively. In dentine, lead ratios in prenatal and postnatal dentine ranging from 2.11 to 3.44

(mean $\pm$ SD=2.73 $\pm$ 0.55) and 2.37 to 63.01 (mean $\pm$ SD=8.36 $\pm$ 13.43), respectively. For all 5 transects, lead ratios gradually increased with age after birth from EDJ to the enamel surface, the highest values being recorded in enamel closest to the crown surface.





**Figure 4.6** Distribution of lead ratios across two teeth for child HT1

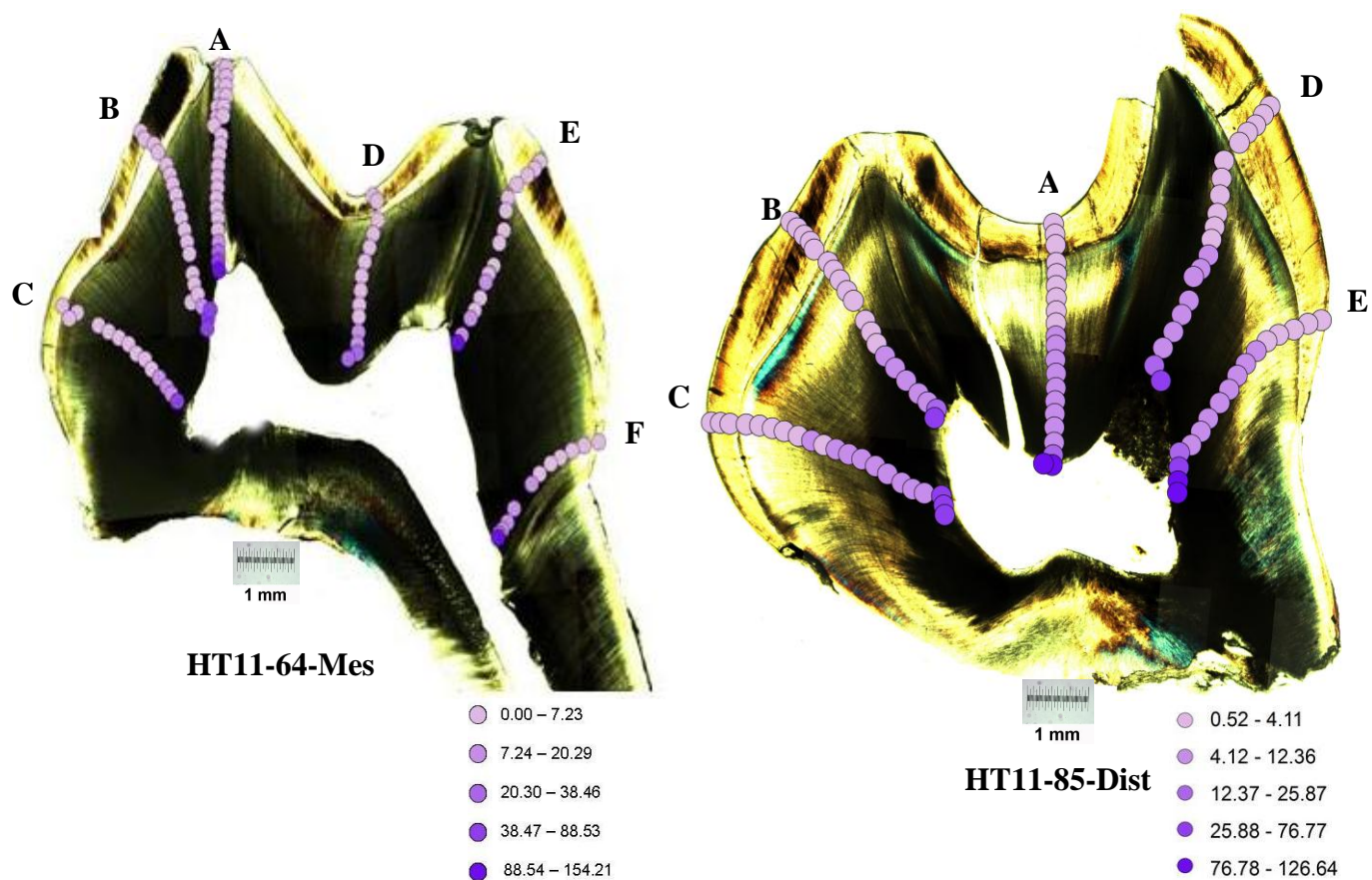


**Figure 4.7** Comparison of dentine lead ratios by age interval in the two teeth for child HT1

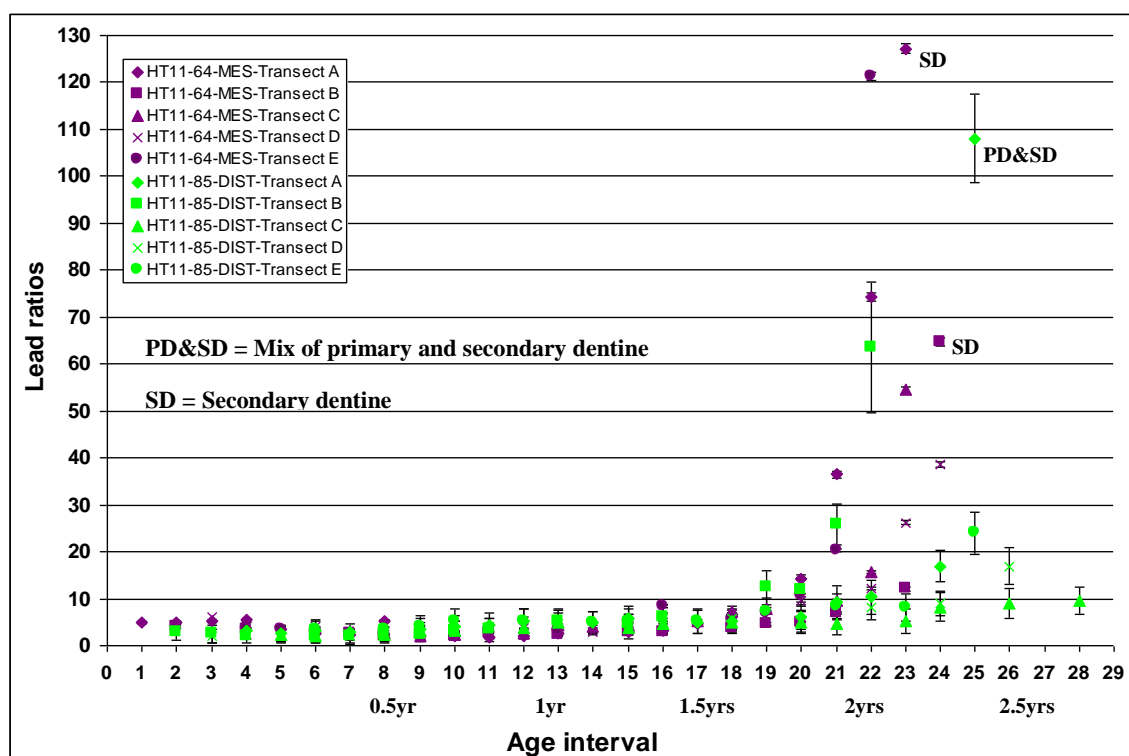
For child HT11 who had the lowest BLL of 0.52 µg/dL, six transects (A-F) were measured across tooth HT11-64, but for transect 'F' age at each ablation point was not measured due to unidentifiable incremental growth lines. Lead ratio distributions in each transect showed the same pattern as child HT1, i.e low ratios observed in enamel, higher ratios in postnatally formed dentine, particularly in the regions close to the pulp cavity. (Figure 4.8 and 4.9) Lead ratios in prenatal enamel between 0.69 and 1.53 (mean±SD=1.09±0.42), and in postnatal enamel between 0.52 and 1.52 (mean±SD=0.91±0.39). Lead ratios in prenatal dentine range 3.47-6.08 (mean±SD=4.7±0.9), lower than in postnatal dentine 1.38-127.20 (mean±SD=12.52±24.28). In tooth HT11-85, lead ratios in postnatal enamel ranging from 0.52 to 3.16 (mean±SD=1.11±0.67), but lead ratios in prenatal enamel were not identified because there were no sampling points in prenatal enamel. Lead ratios in prenatal dentine ranged from 1.95-3.21 (mean±SD=2.58±0.53), which are lower than those in postnatal dentine 1.96-108.03 (mean±SD=8.28±14.62).

Average lead ratios together with the age range of enamel formation were measured for each tooth from children HT1 and HT11. Comparatively low ratios were determined in prenatal enamel and higher ratios were found in postnatal enamel. For each tooth, it is difficult to identify a trend of lead ratios along the prisms due to the small number of sampling points in enamel. However a tendency for these values to increase from the EDJ to the enamel surface was found in some teeth. The age ranges for enamel formation at the sampling points ranged from 146 days (5 months) before birth to 247 days (8 months) after birth. In child HT11 tooth 64, average enamel lead ratios were slightly higher than those in the other tooth (tooth 85) from the same child. However, no significant differences between lead ratios in enamel for the pairs of teeth from each child were found. In dentine, comparatively low ratios were determined in prenatal dentine and higher ratios were found in postnatal dentine. The age ranges for the formation of dentine ranged from 185 days (~6 months) before birth to 1,033 days (~3 years) after birth.

Overall in the initial four teeth, lead ratio distributions in each transect showed a similar pattern, with comparatively low ratios of lead observed in enamel, higher ratios in postnatally formed dentine, with the highest ratios in the regions close to the pulp chamber. Regarding ages of exposure, dentine provided a record of exposure over a longer period than enamel. Based on these findings, dentine was identified as the more promising biomarker for identifying the chronological record of early life exposure.



**Figure 4.8** Distribution of lead ratios across two teeth for child HT11



**Figure 4.9** Comparison of dentine lead ratios by age interval in the two teeth for child HT11

## Summary

**Question 2a:** Should we sample enamel and/or dentine?

**Answer:** Dentine provides the best opportunity to explore the detailed chronology of lead exposure as it incorporates higher levels of lead and represents a longer period of exposure than enamel.

### 4.3.2 Question 2b: How many transects per tooth are required?

In enamel, Humphrey and colleagues (2008) demonstrated that calcium normalised strontium intensities at the same age varied depending upon where samples were located in the crown of the tooth. [245] This is due to the gradients of enamel mineralisation across the tooth crown. [245, 324] They also determined that a reliable chronology could be determined when controlling for distance from the surface of the tooth. [324] Dentine and enamel mineralise differently but dentine also continues to mineralise after its initial formation. The second step in answering question 2 was to determine whether sampling pits in dentine which sampled the same age of formation had similar levels of lead and whether location in the coronal dentine needed to be taken into account when analysing the data; i.e. to determine how many transects were required for each tooth to give a reliable signal of lead exposure history.

To test the extent of within-tooth trace element variation, five ablation transects were made across two teeth from each child (HT1-54,55 and HT11-64,85), orientated to follow dentine tubules from the EDJ to the pulp cavity, from all regions within the tooth crown. Lead ratios in dentine were measured along these transects from the crown to the pulp cavity. My focus was on coronal dentine (the dentine under the tooth crown) which consists principally of primary dentine, so the results are presented mainly for primary dentine as show in Figure 4.10 and 4.11. However, I also identified secondary dentine in some tooth sections.

The results showed consistent lead ratios at the same age interval in dentine, irrespective of where the transect was within the tooth. Comparatively higher lead ratios were found in postnatal dentine than in prenatal dentine but when aligned by age interval, lead ratios across transects were consistent, with all transects showing a steady increase in lead ratio with age, with lead ratios rapidly increasing approaching the pulp cavity. A comparison

of lead ratios in each transect across the primary dentine in both teeth from each child shows the same pattern, see Figure 4.10 and 4.11 (these figures show the same data as Figure 4.7 and 4.9 but focus on primary dentine, allowing the Y axis to be stretched to show finer details).

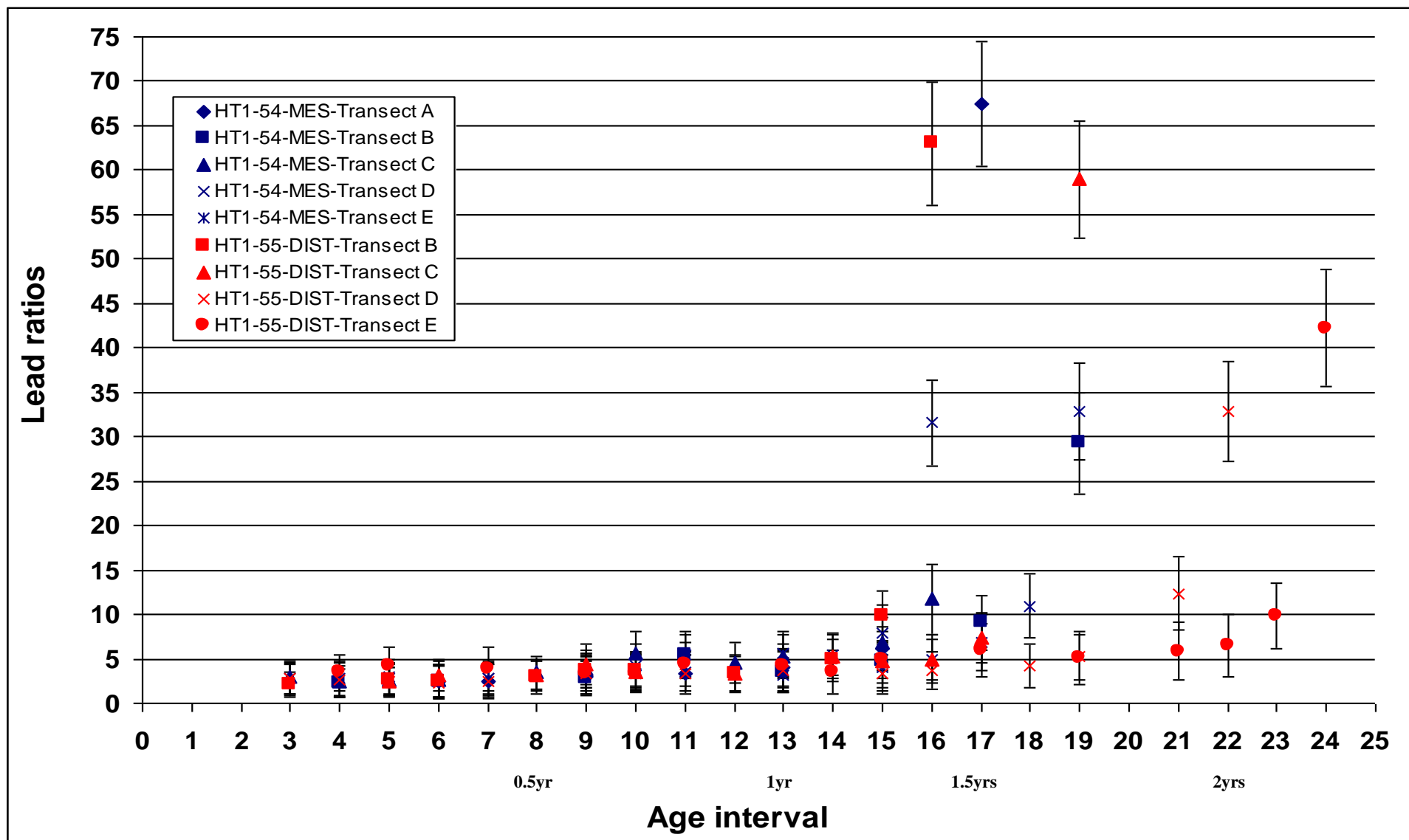
For child HT1 tooth 54, the average lead ratio in primary dentine before birth was 2.7, and this slightly increased to 5.9 at 430-472 days (1 year 3 months) after birth before significantly increasing almost fivefold to 27.8 at 514-556 days (1.5 years) after birth. For child HT1 tooth 55, the average lead ratio in dentine before birth was 2.8, which slightly increased to 5.7 between 430 and 472 days (1 year 3 months) after birth, and then increased markedly to 42 at the age of the ablation pit between 808-850 days (2 years 4 months) after birth. In this child, the largest variation in measurement error was found in the primary dentine close to the pulp cavity, and in secondary dentine.

For child HT11, tooth 64, average lead ratios in primary dentine before birth were 4.7, increased slightly to 6.5 until 598-640 days (1 year 9 months) after birth, before gradually increasing after 2 years. For child HT11 tooth 85, the average lead ratio in primary dentine was around 2.6 before birth, then slightly increasing to 4.2 until 600 days (1 year 8 months) after birth and significantly increasing after that until 2.5 years after birth.

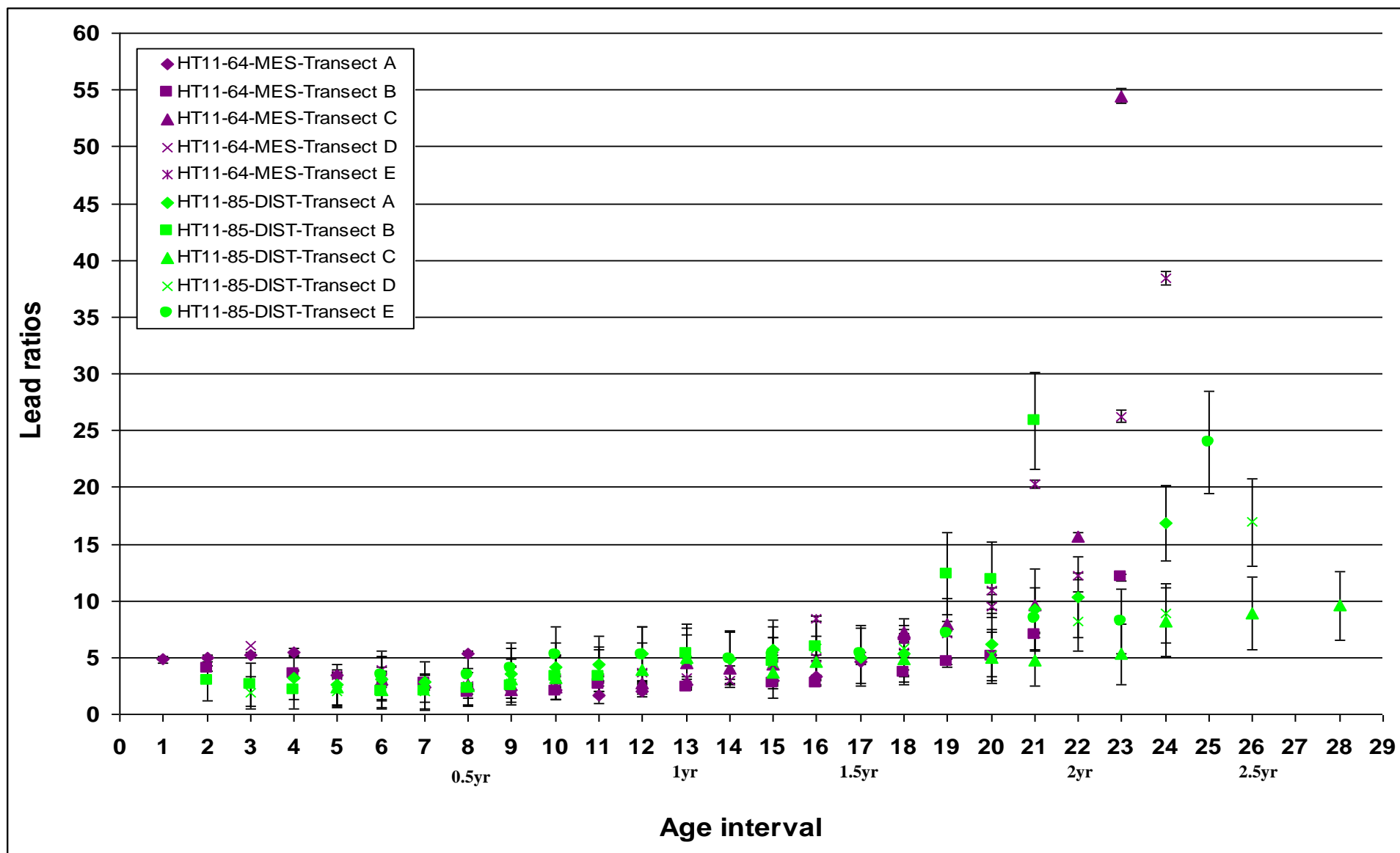
The results for both children indicate that lead ratios in primary dentine are consistent in teeth from the same child at the same age interval, and slightly increase with age after birth in both children, as shown in most transects. Higher ratios were found around the dentine close to the pulp cavity. Mean levels of error for lead ablation points in dentine are higher than those in enamel, however, levels of error are still relatively low ( $<4$ ).

From these results and associated histological data it was evident that individual transects corresponded to different postnatal periods after birth. However, irrespective of the age period covered by each transect, the lead ratios at the same age interval remained similar. Although lead ratios in primary dentine in the initial four teeth were low, the ratios show consistent characteristics, with the profile for lead being relatively flat apart from the postnatal dentine close to the pulp cavity, and show no significant changes in concentration with age.

Given the consistent patterns in lead ratios at the same age interval across two teeth from each child, we decided that for the remaining pairs of teeth only one transect per tooth was required to demonstrate the magnitude and temporal changes in lead ratios.



**Figure 4.10** A comparison of lead ratios in primary dentine in two teeth from child HT1



**Figure 4.11** A comparison of lead ratios in primary dentine in two teeth from child HT11



## Summary

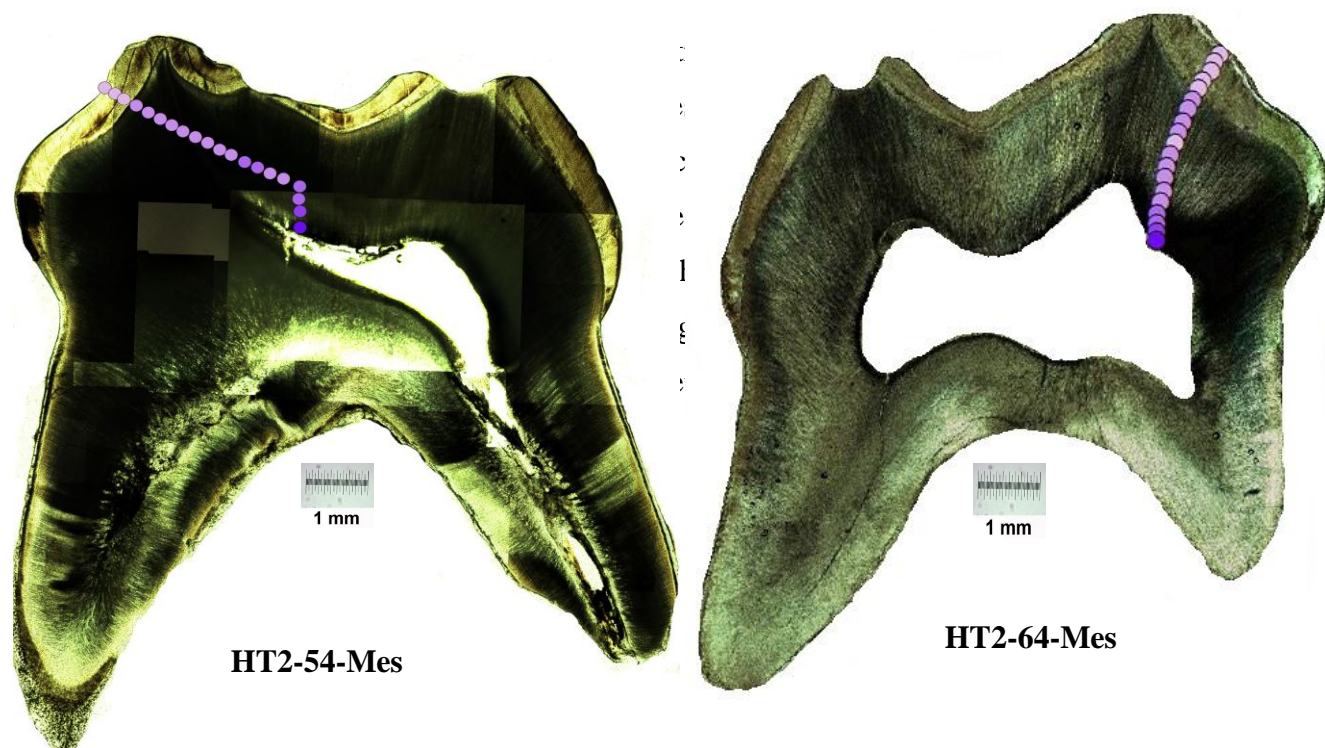
**Question 2b:** How many transects per tooth are required?

**Answer:** Based on results in a small set of good tooth sections, in primary dentine, one transect may provide the pattern of lead ratios which are consistent at the same age within each tooth, indicating that the history of lead exposure may be determined using a single, multi-point ablation transect on longitudinal sections of individual teeth. However, we recommend further validation this finding in larger population samples, including populations with known lead exposure.

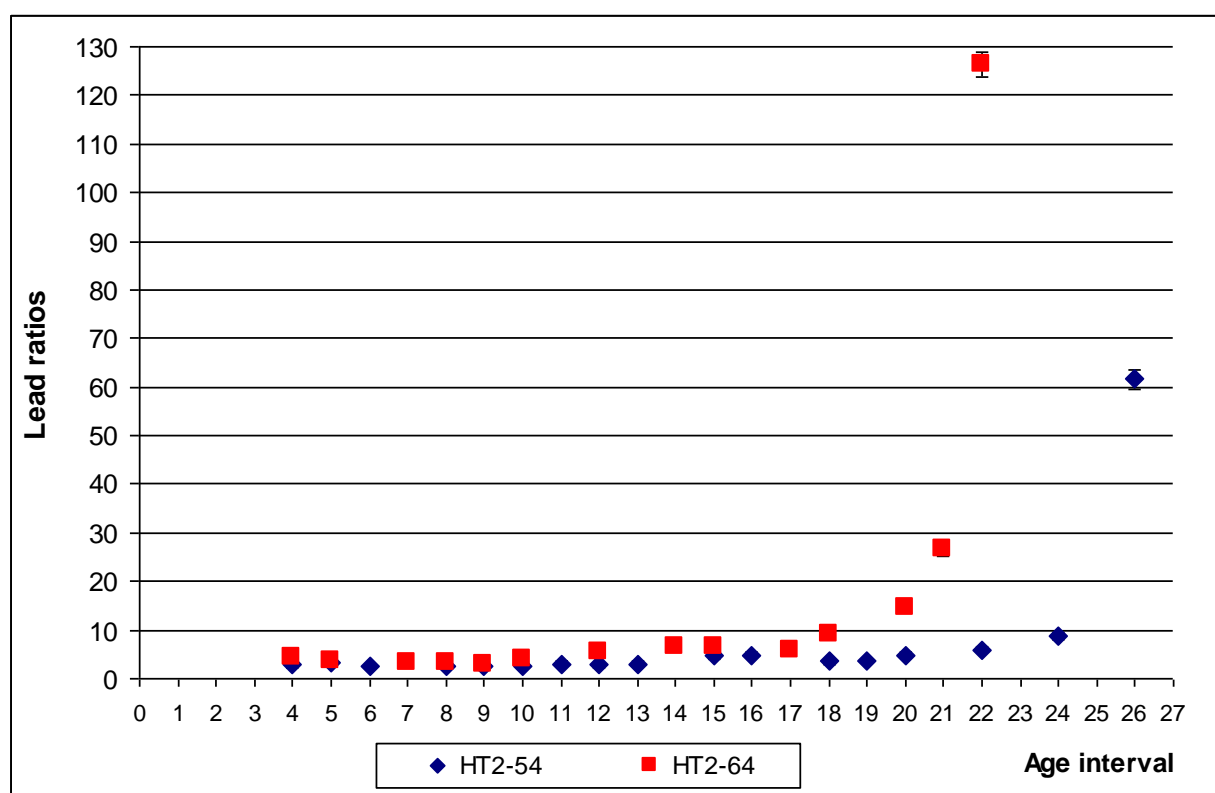
### 4.3.3 Question 2c: How many teeth per child are required?

Preliminary results from the initial four teeth indicated that consistent lead ratios at the same age interval were found in primary dentine, although this pattern was less consistent closer to the pulp cavity where lead ratios increased rapidly. Based on these trends, only one transect was ablated in the 16 remaining teeth in order to further explore the value of lead ratios in dentine as a biomarker of the history of lead exposure. Although I collected two teeth for each child, it was not possible to measure the age of ablation pits for child HT8 tooth 84 (HT8-84) due to poor section quality.

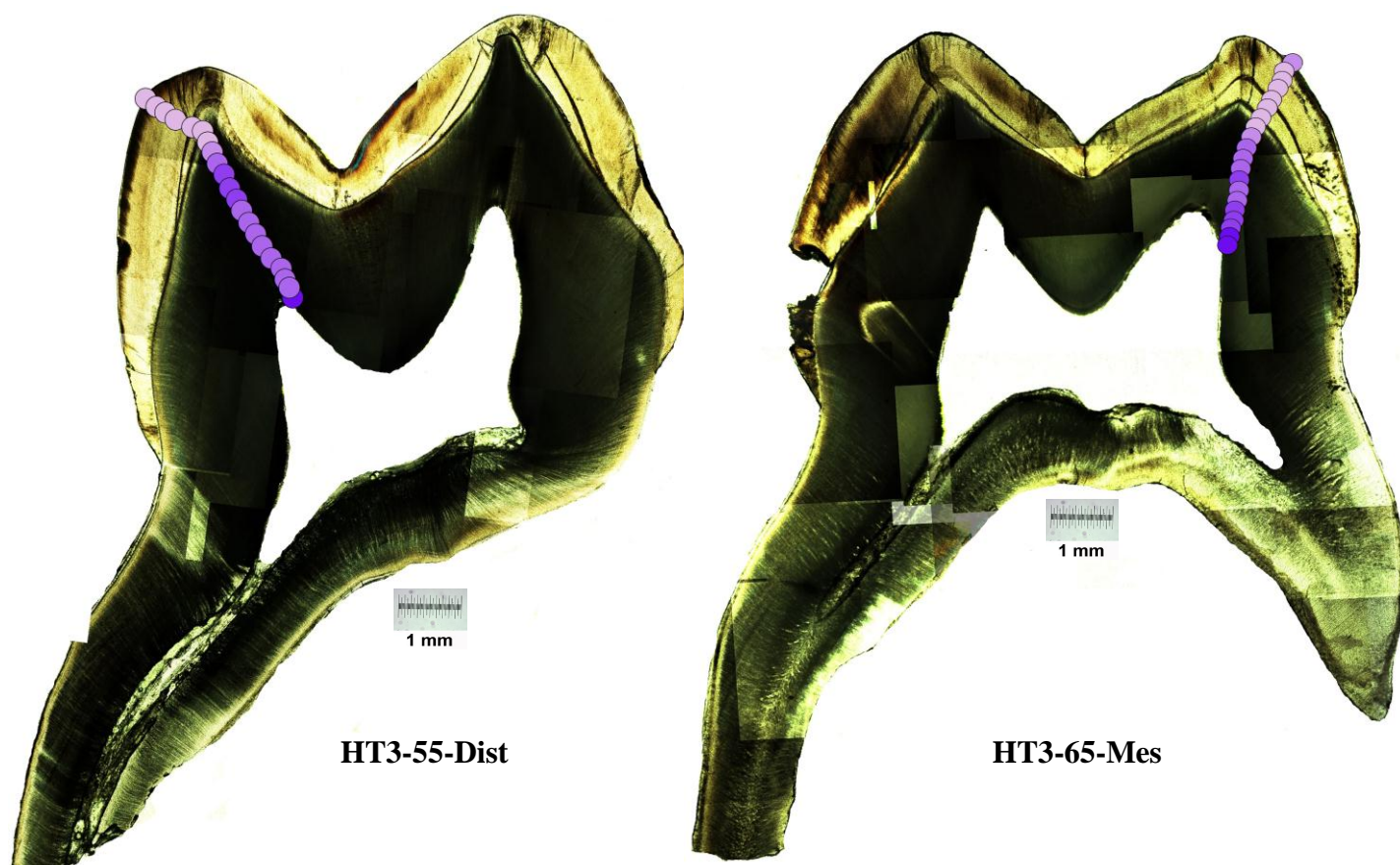
For the 15 remaining teeth with histological data, the age ranges for dentine formation at the sampled points ranged from 98 days (~3 months) before birth to 1,102 days (~3 years) after birth. The average measurement error was low except in the two teeth from child HT3, discussed further below.



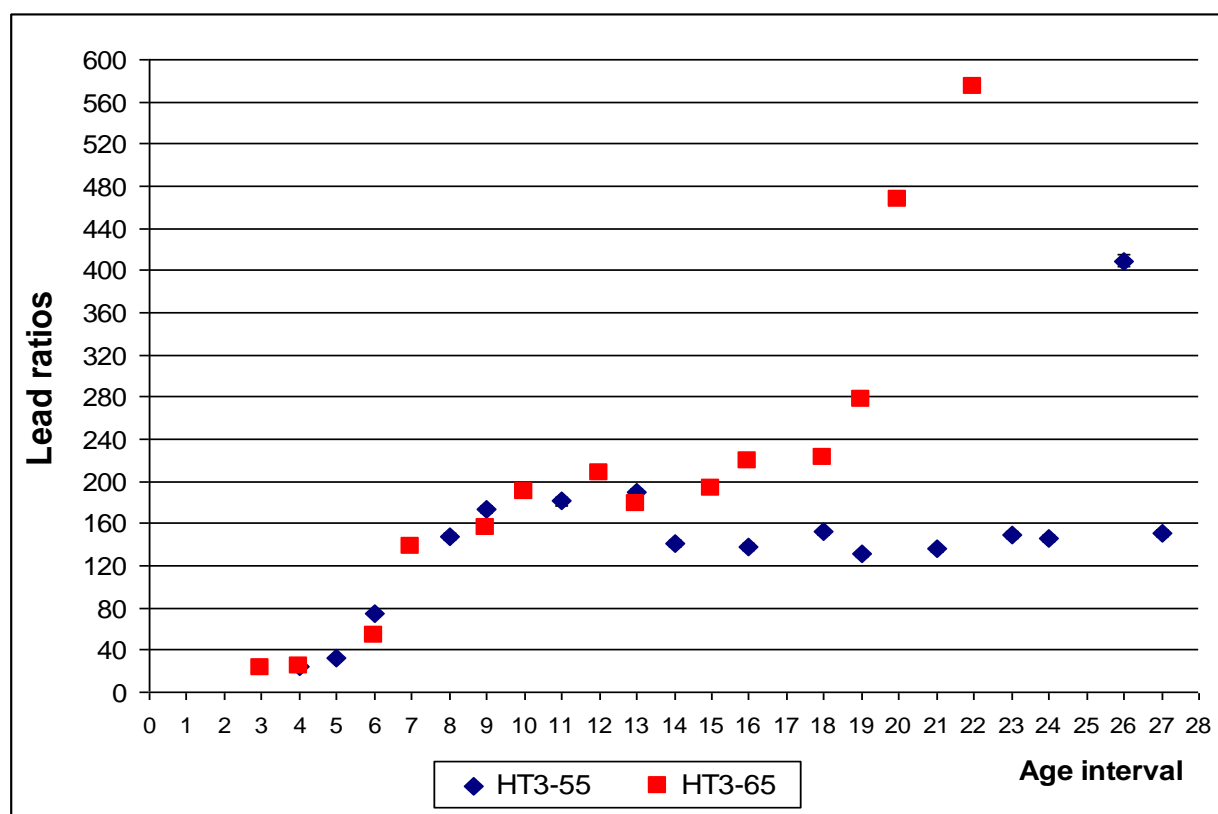
**Figure 4.12** Lead ratio distribution for the two teeth from child HT2



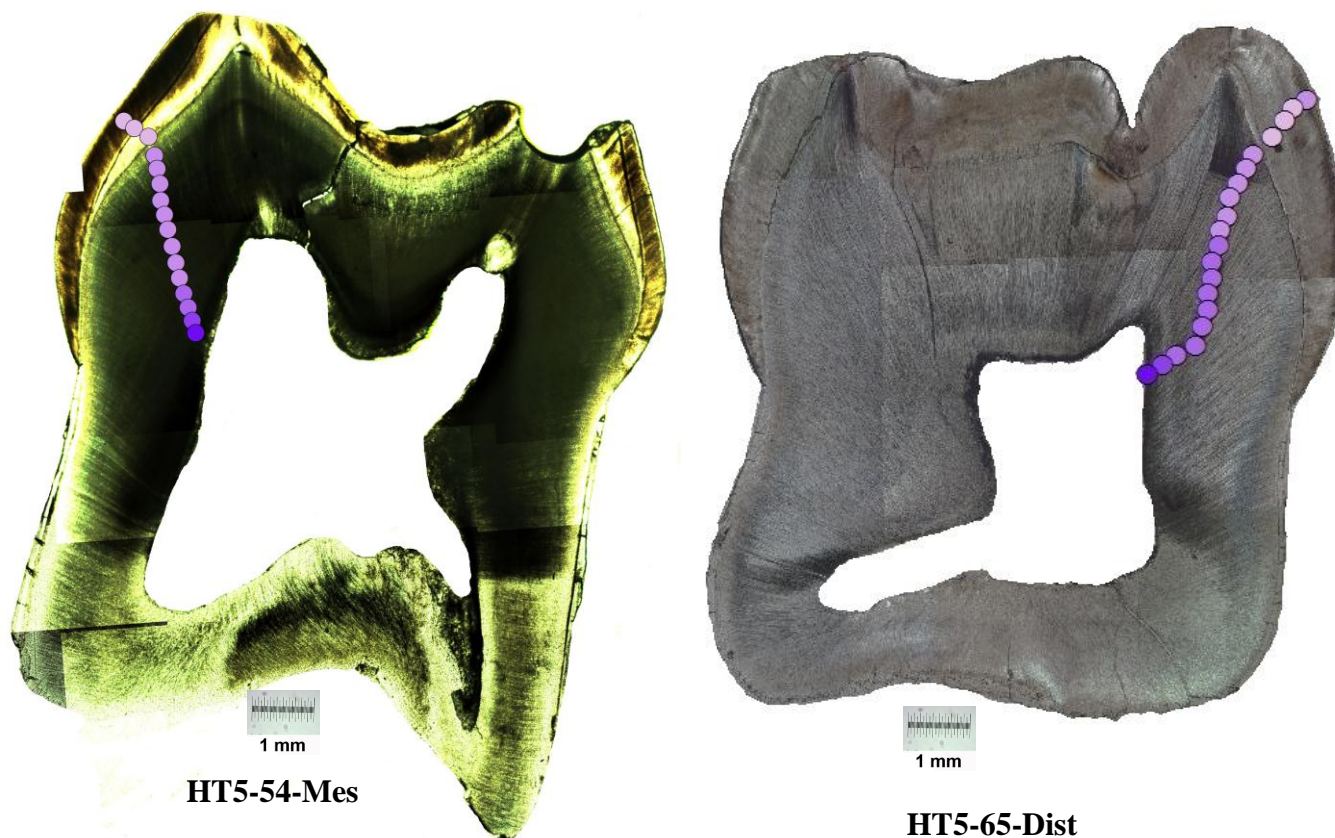
**Figure 4.13** Lead ratios in primary dentine for two teeth from child HT2



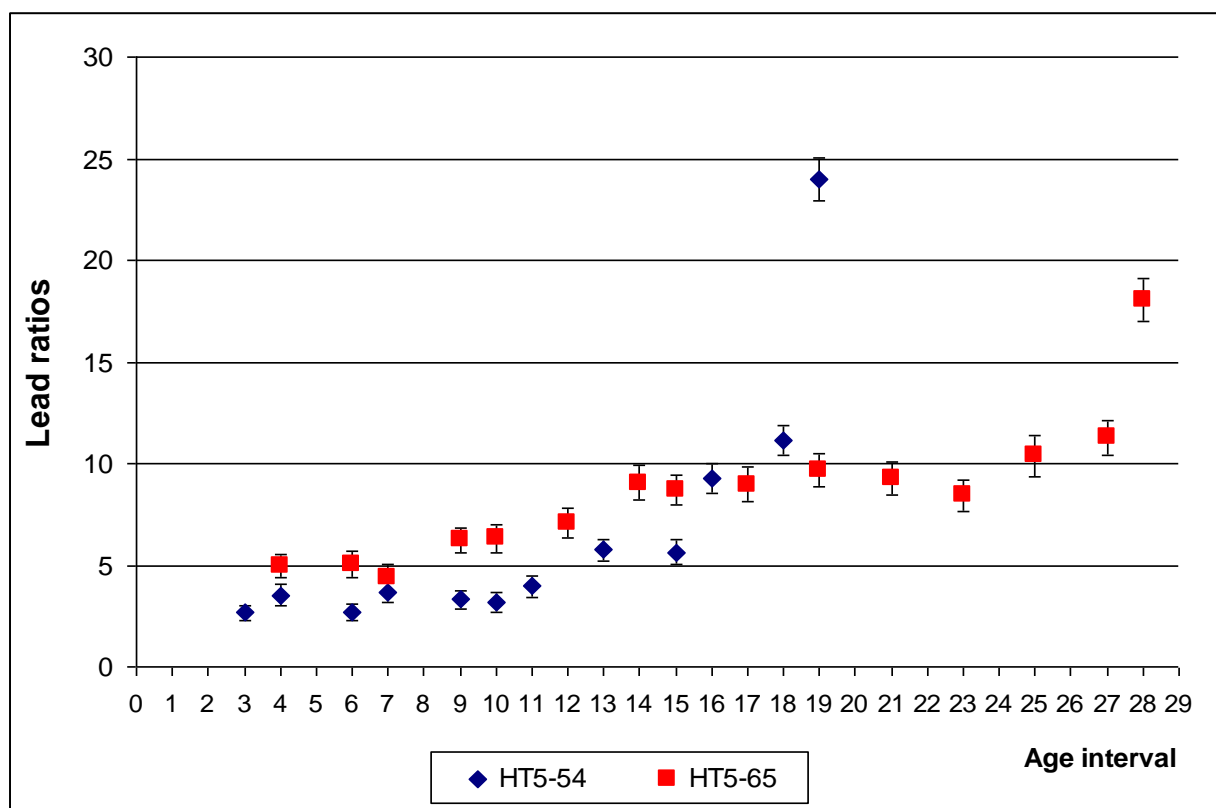
**Figure 4.14** Lead ratio distribution for the two teeth from child HT3



**Figure 4.15** Lead ratios in primary dentine for two teeth from child HT3

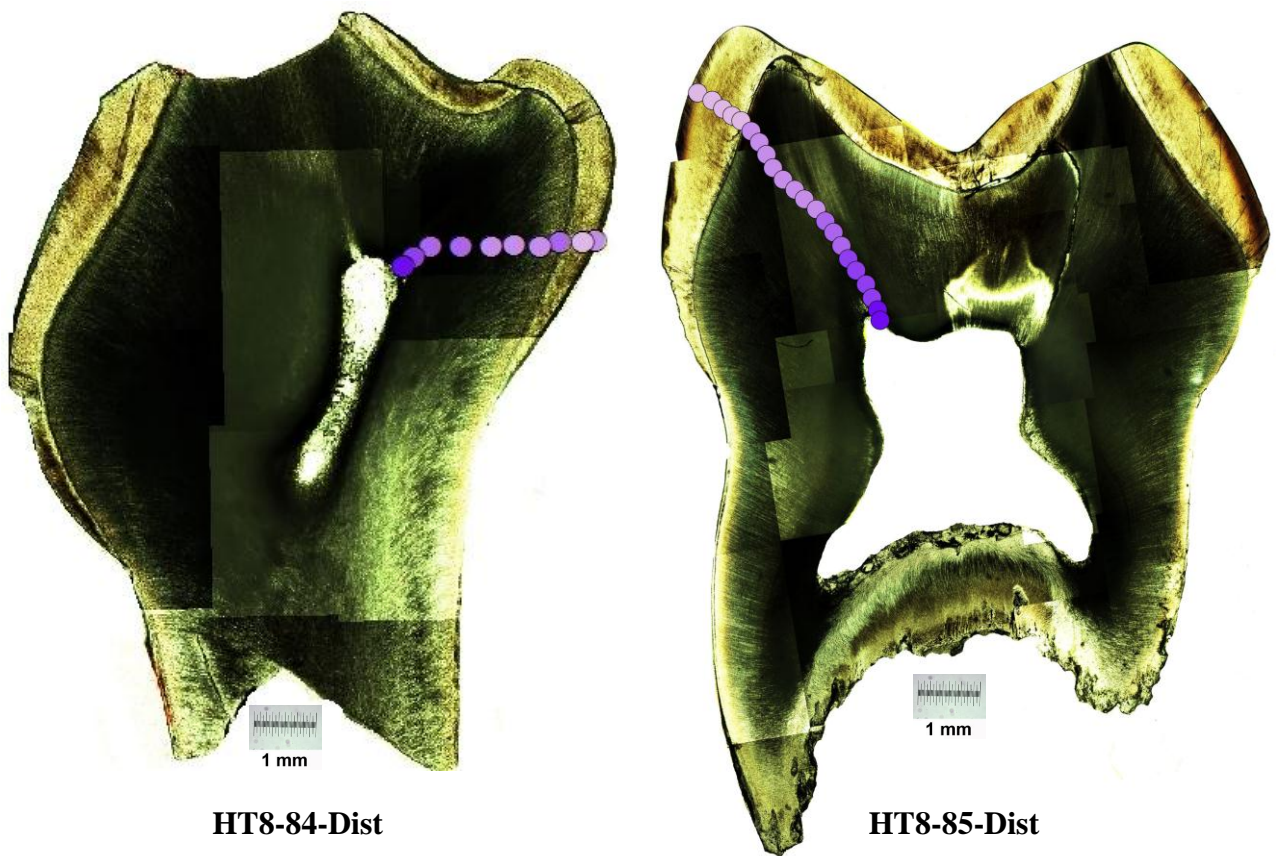


**Figure 4.16** Lead ratio distribution for the two teeth from child HT5

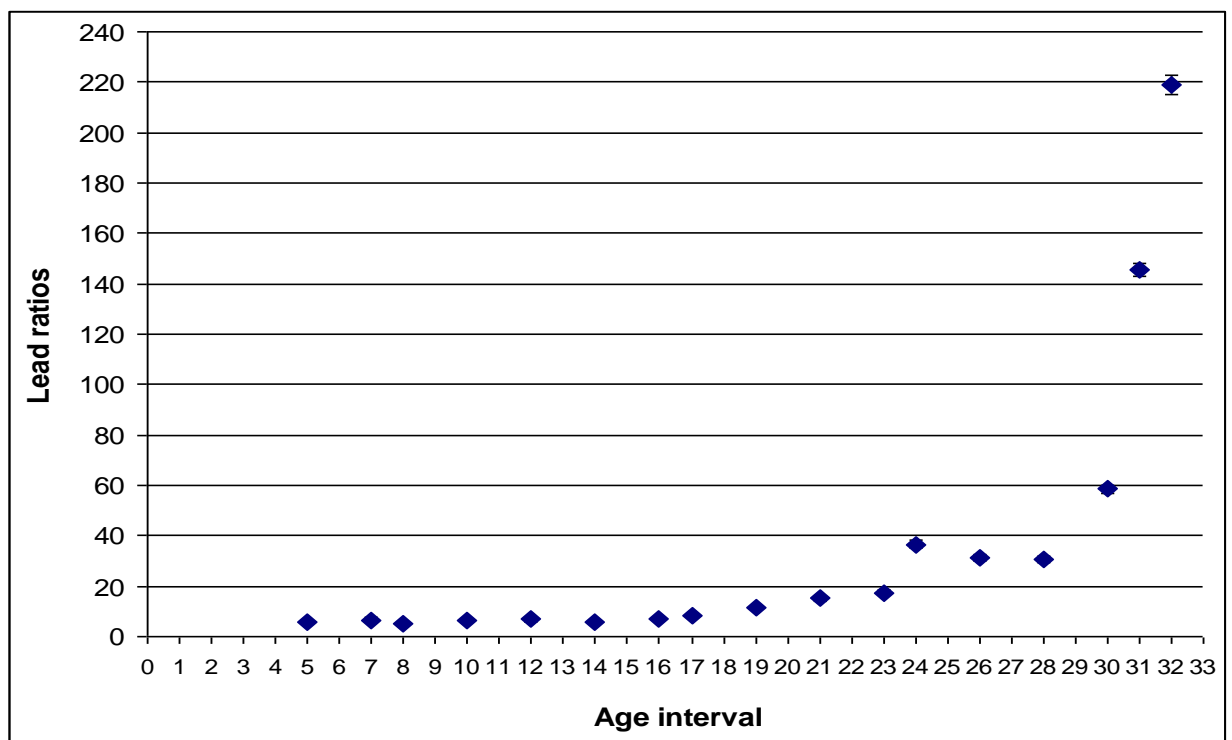


**Figure 4.17** Lead ratios in primary dentine for two teeth from child HT5

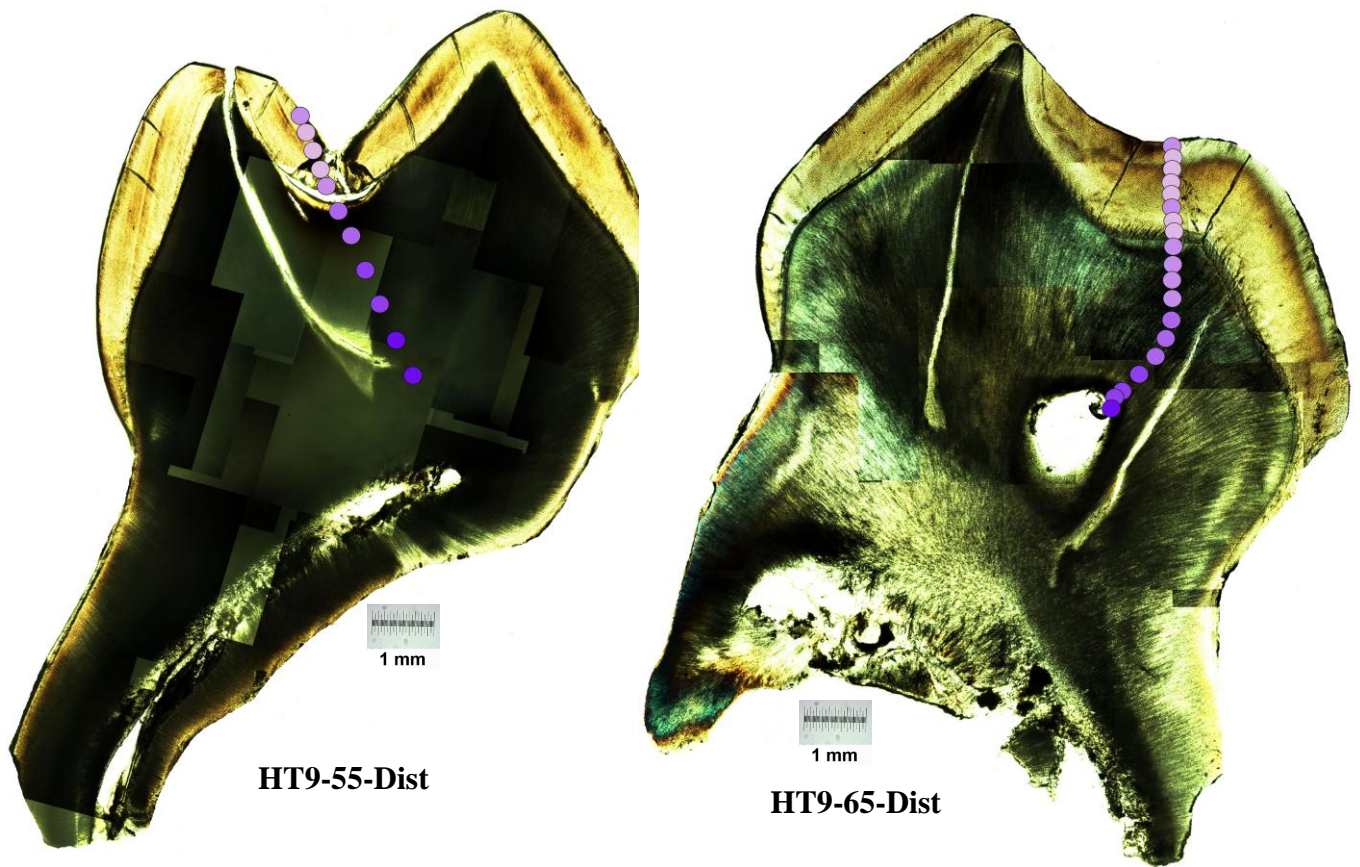




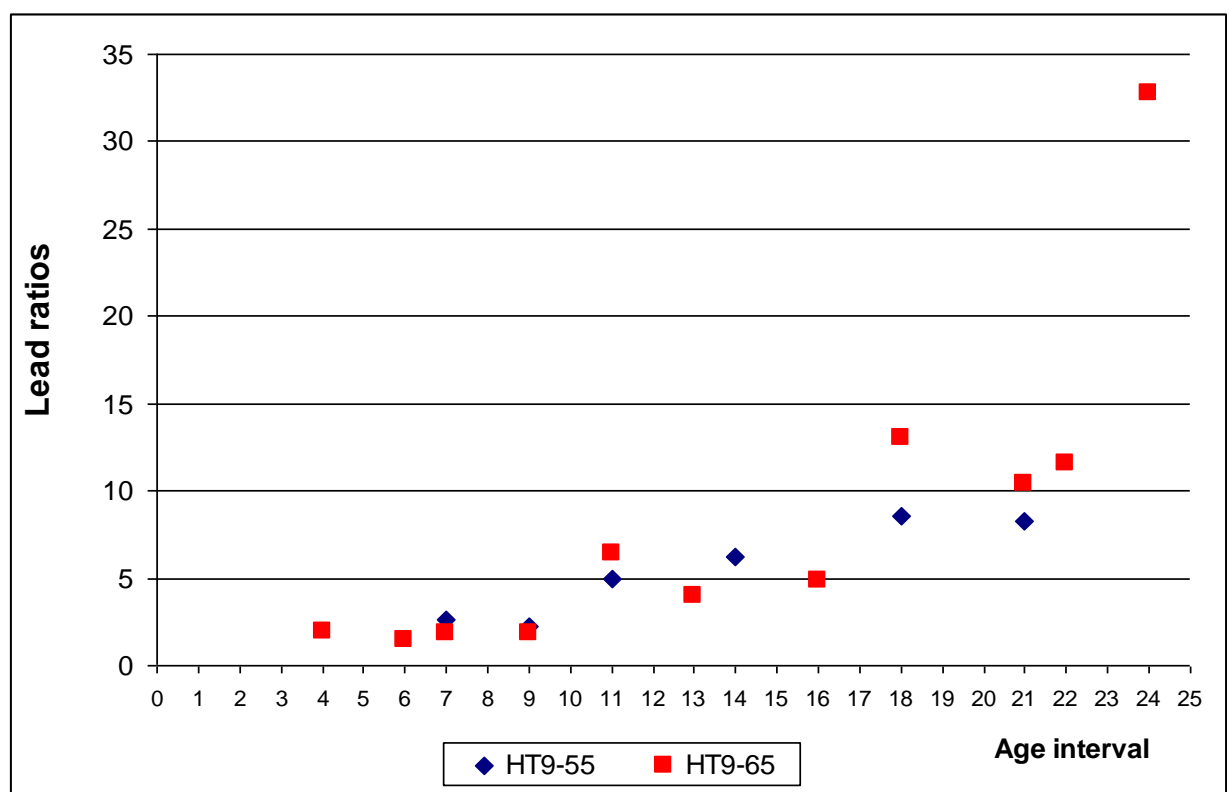
**Figure 4.18** Lead ratio distribution for the two teeth from child HT8



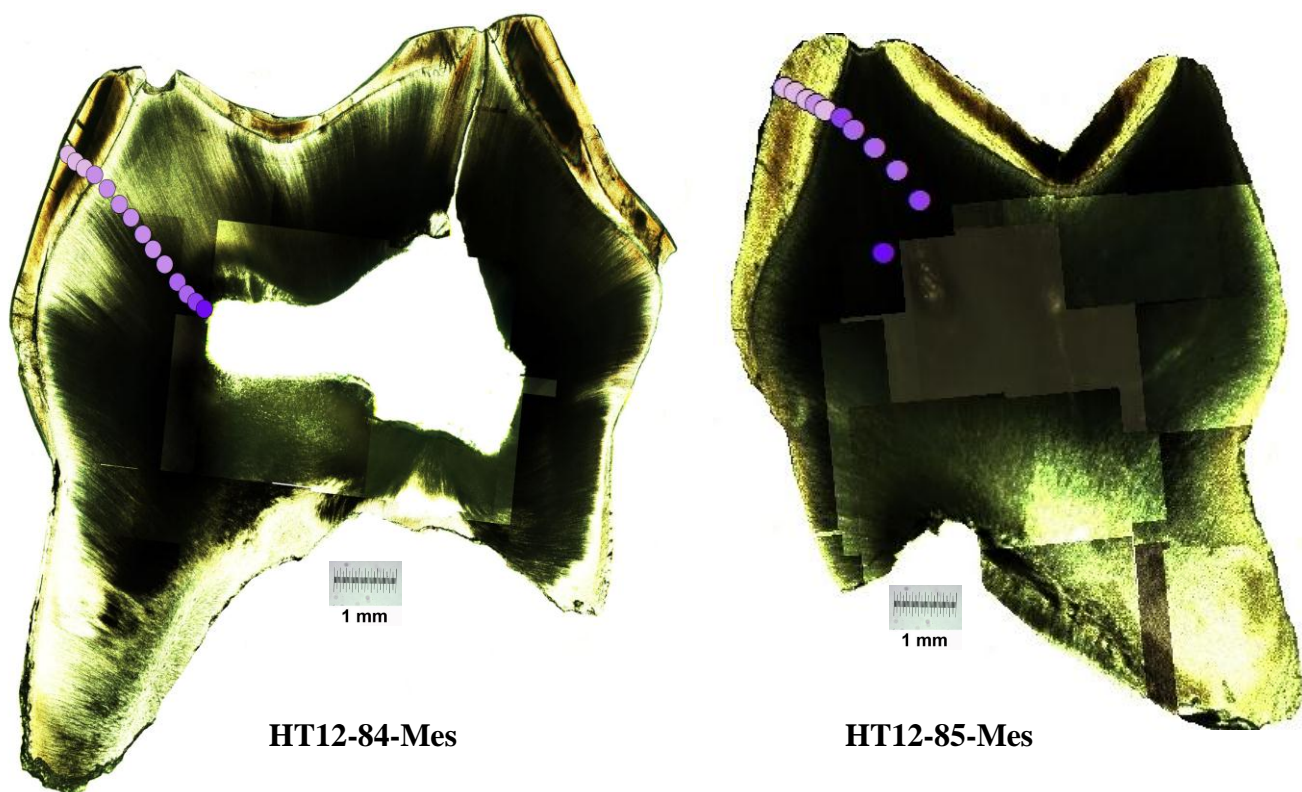
**Figure 4.19** Lead ratios in primary dentine for child HT8 tooth 85



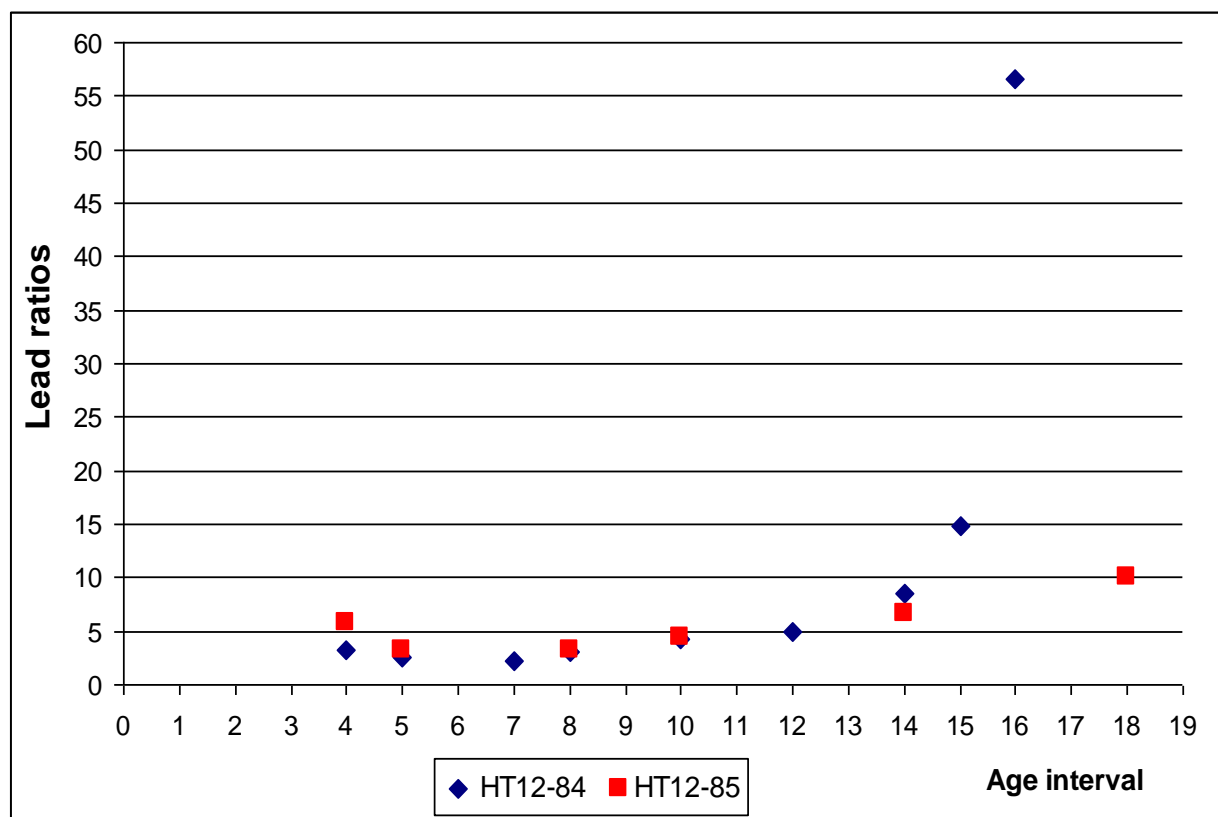
**Figure 4.20** Lead ratio distribution for the two teeth from child HT9



**Figure 4.21** Lead ratios in primary dentine for two teeth from child HT9

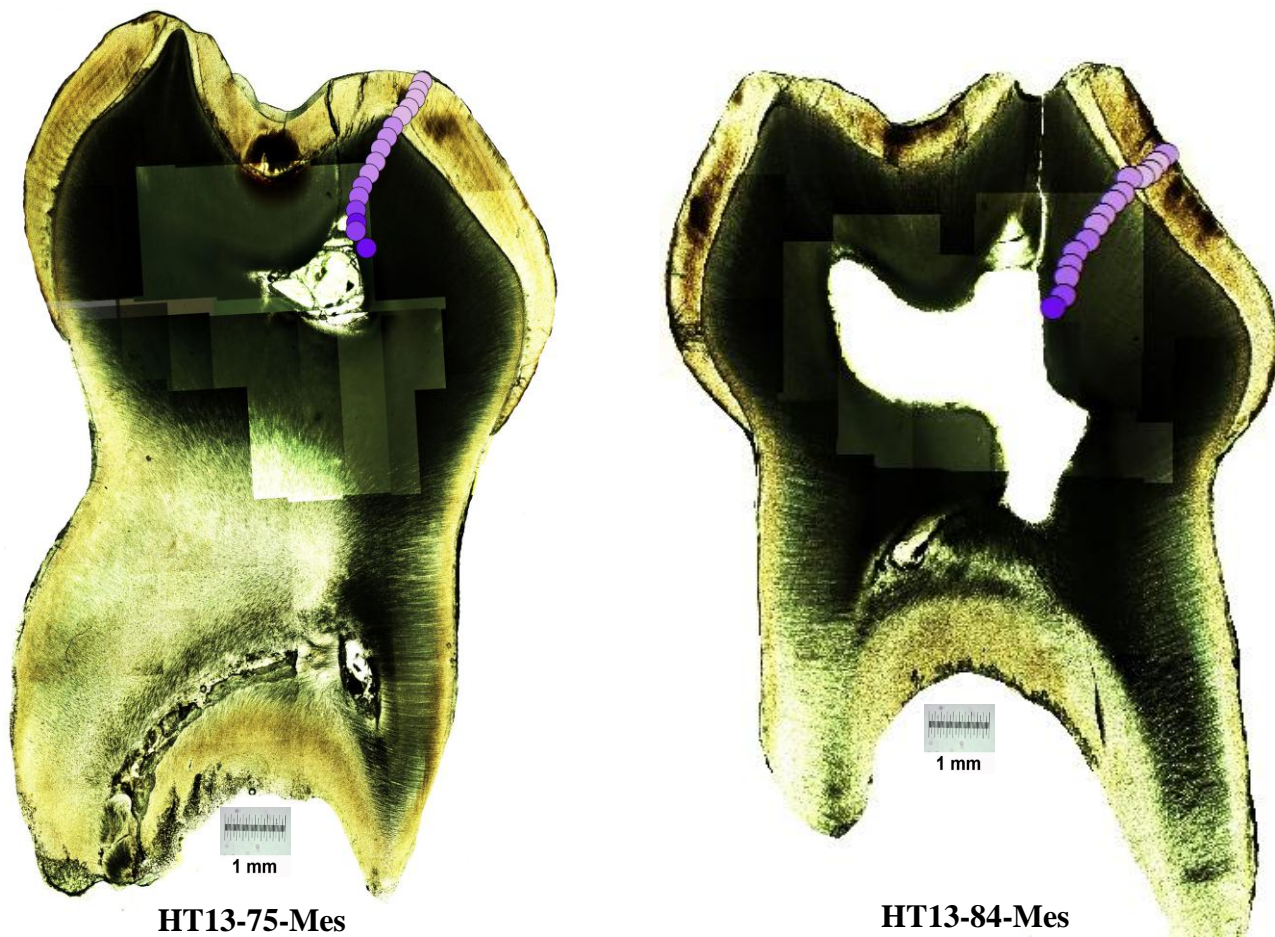


**Figure 4.22** Lead ratio distribution for the two teeth from child HT12

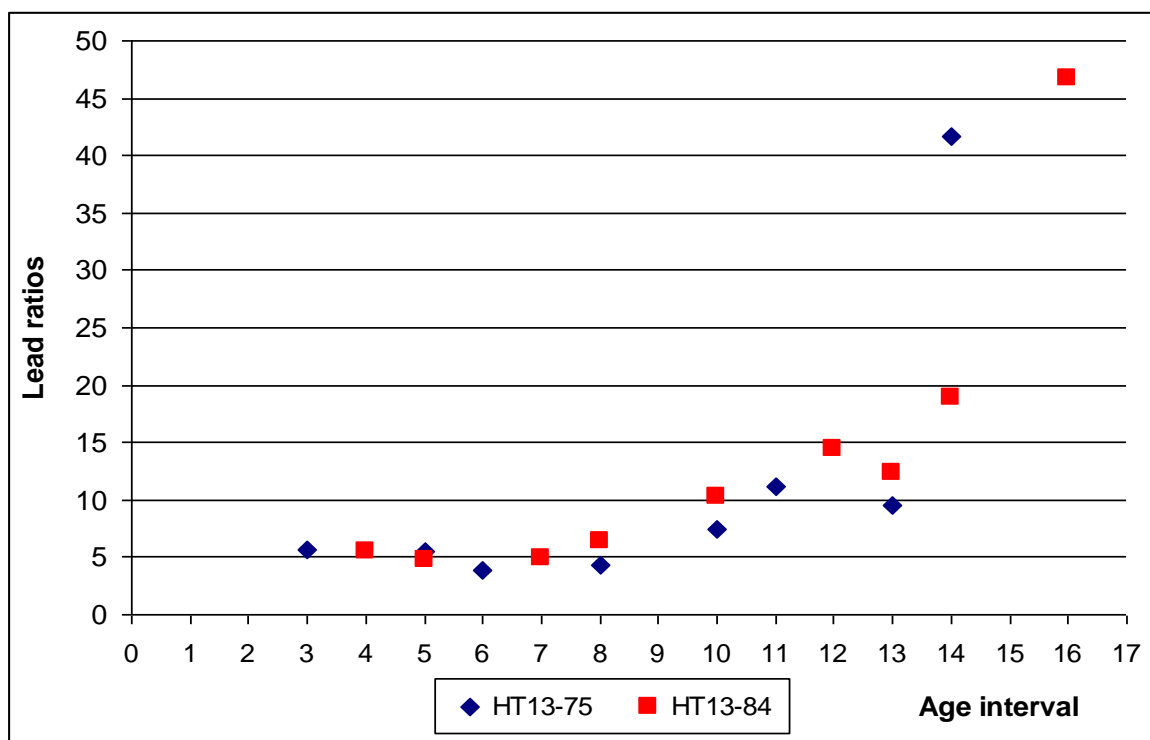


**Figure 4.23** Lead ratios in primary dentine for two teeth from child HT12



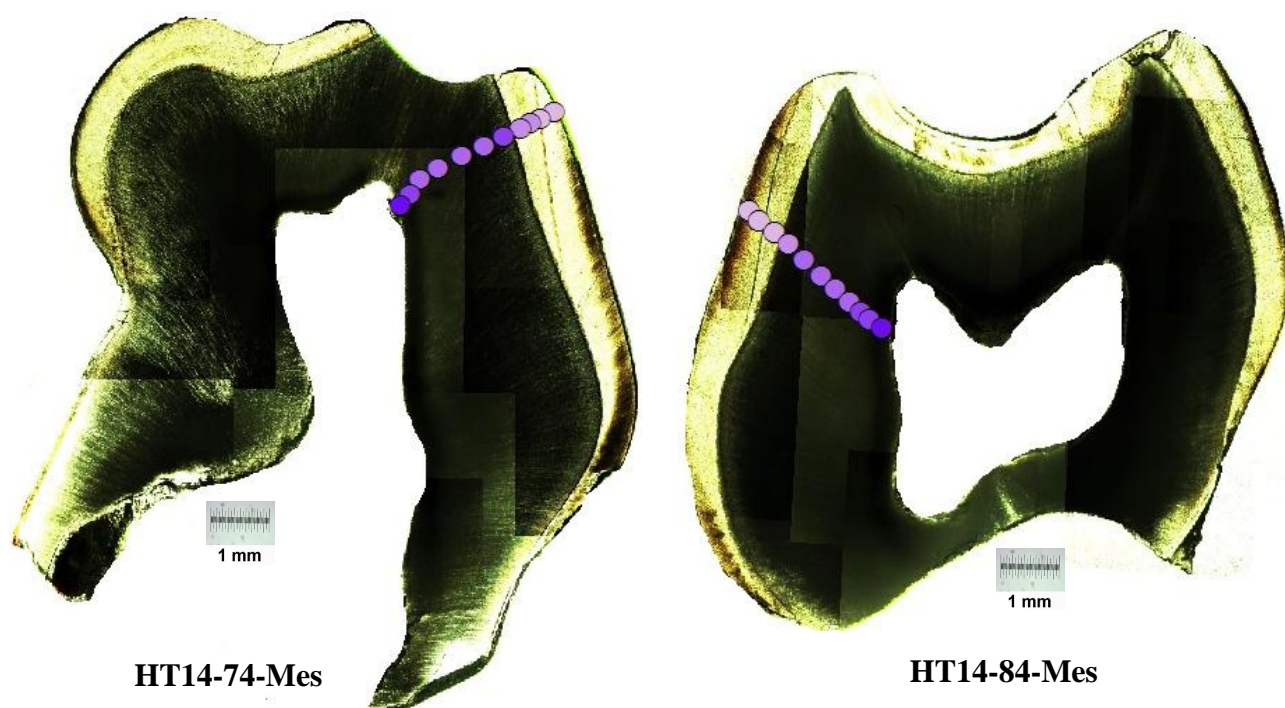


**Figure 4.24** Lead ratio distribution for the two teeth from child HT13

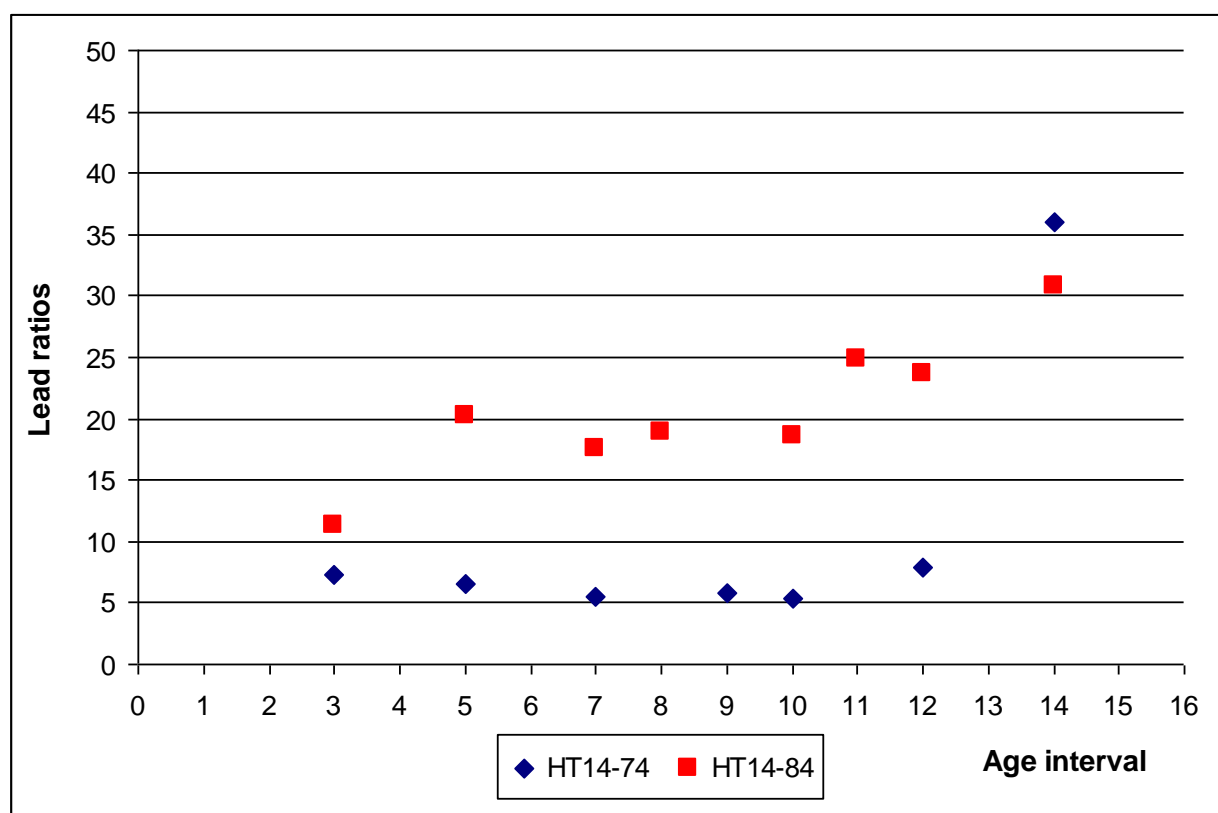


**Figure 4.25** Lead ratios in primary dentine for two teeth from child HT13





**Figure 4.26** Lead ratio distribution for the two teeth from child HT14



**Figure 4.27** Lead ratios in primary dentine for the two teeth from child HT14

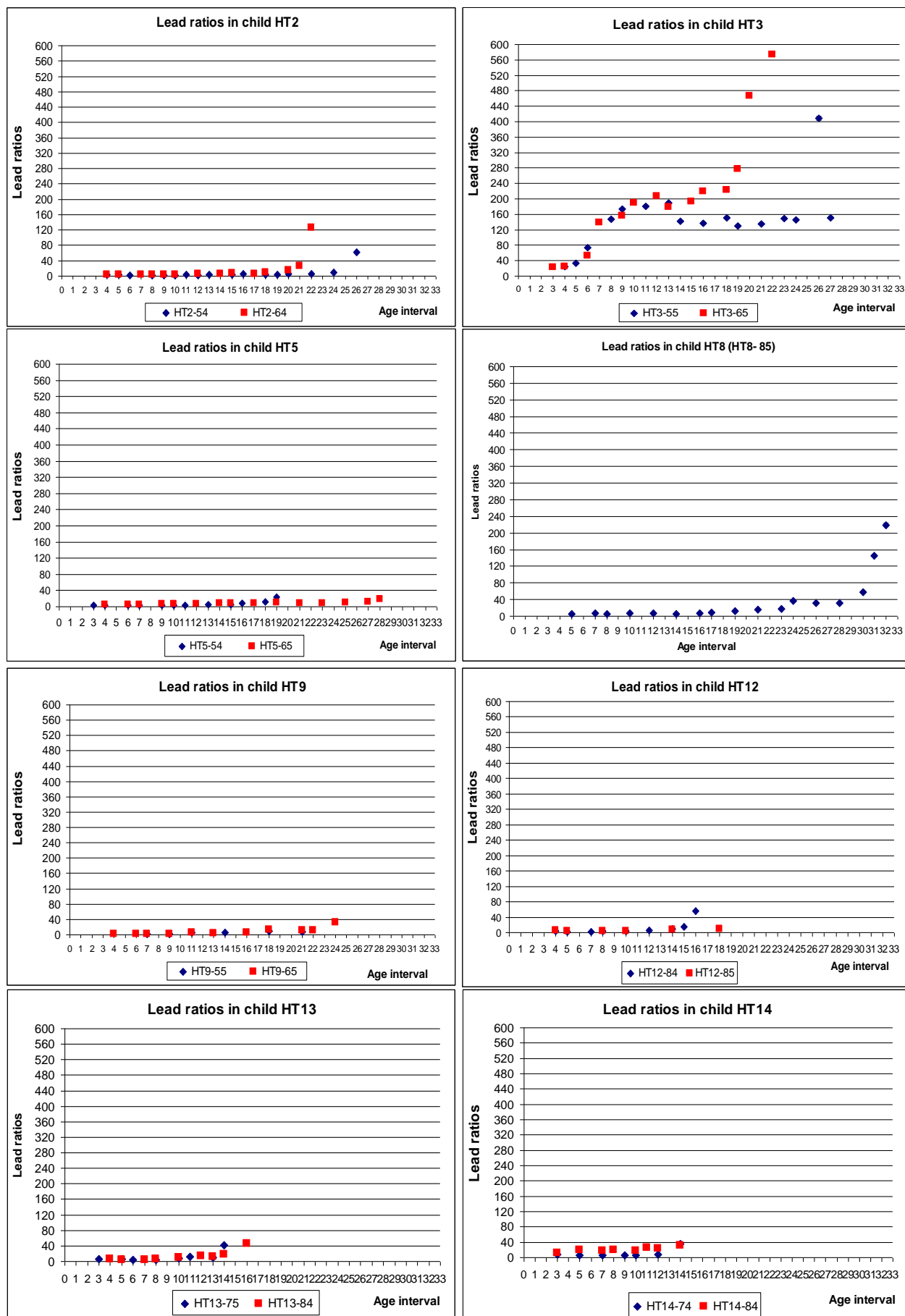
As shown in Figure 4.28 with the X axis scaled to the same values for all teeth pairs, little difference was seen between lead ratios sampled in the primary dentine at the same age interval for the two teeth from the same child with exception of child HT3 and HT14, indicating that one transect in one tooth is probably sufficient to recreate patterns of lead incorporation over the child's early years, although pairs showed continue to be studied until we know more about this inter-tooth variability.

With respect to inter-child variability, we found that the majority of the transects from each child showed a consistent pattern in dentine of low lead ratios that slightly increased by age interval, with the highest lead ratios being found in the dentine near the pulp cavity. However, the two teeth from child HT3 did not follow this pattern (Figure 4.15). Lead ratios in primary dentine for this child increased until interval 304-346 days (8 months) after birth, then they slightly decreased around one year after birth, and afterwards significantly increased moving towards the pulp cavity. These reproducible changes in lead ratios can be traced in both deciduous molars (HT3-55, 65) from birth to dentine pulp cavity. This lead distribution seems likely to be due to lead exposure experienced by this child during their early life, and therefore suggests that dentine analysed using this technique may be useful in identifying the history of lead exposure.

## **Summary**

**Question 2c:** How many teeth per child are required?

**Answer:** The results showed that differences in lead ratios between good sections in two teeth from the same child were small although we do not provide sufficient evidence that one tooth is sufficient. We recommend an expansion of this study with a larger number of samples and the collection of multiple teeth from each of the participants to allow refinement and confirmation of the histological technique proposed in the present study.



**Figure 4.28** Comparison of primary dentine lead ratios of all samples from two teeth each child

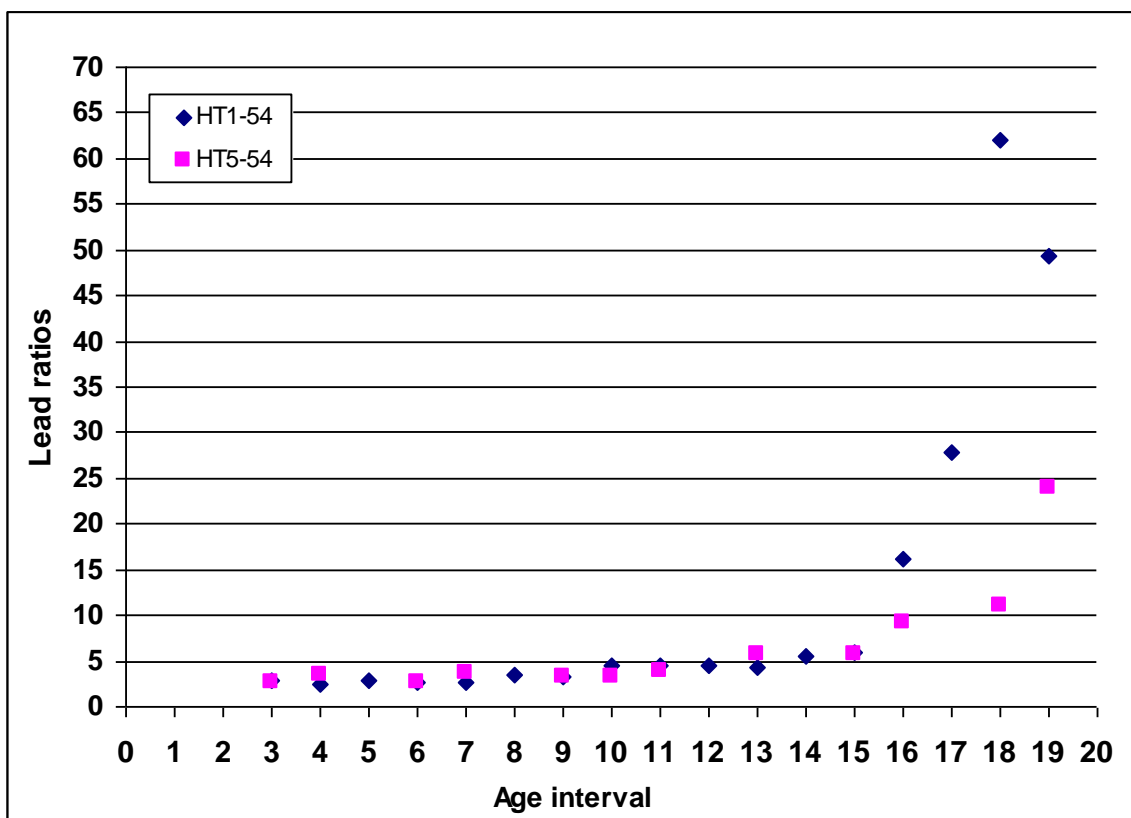
#### **4.3.4 Question 2d: Does tooth type matter?**

A comparison between lead ratios in primary dentine from different children but the same tooth type (tooth types 54, 55, 64, 65, 84 and 85) was made. The same trend of steadily increasing lead ratios with age interval and a rapid increase upon approaching the pulp cavity was found among matched tooth types as shown in Figures 4.29-4.34. Significantly different lead ratios across two children who had tooth type 55 and 65 were found, although this is probably because primary dentine lead ratios in HT3 were >100 times higher than those in other children irrespective of tooth type. In addition, there was some variation in lead ratios at the same age interval across the three children who had donated tooth type 84 (Figure 4.33), although the trend of increasing lead ratios with age interval was seen in all three teeth.

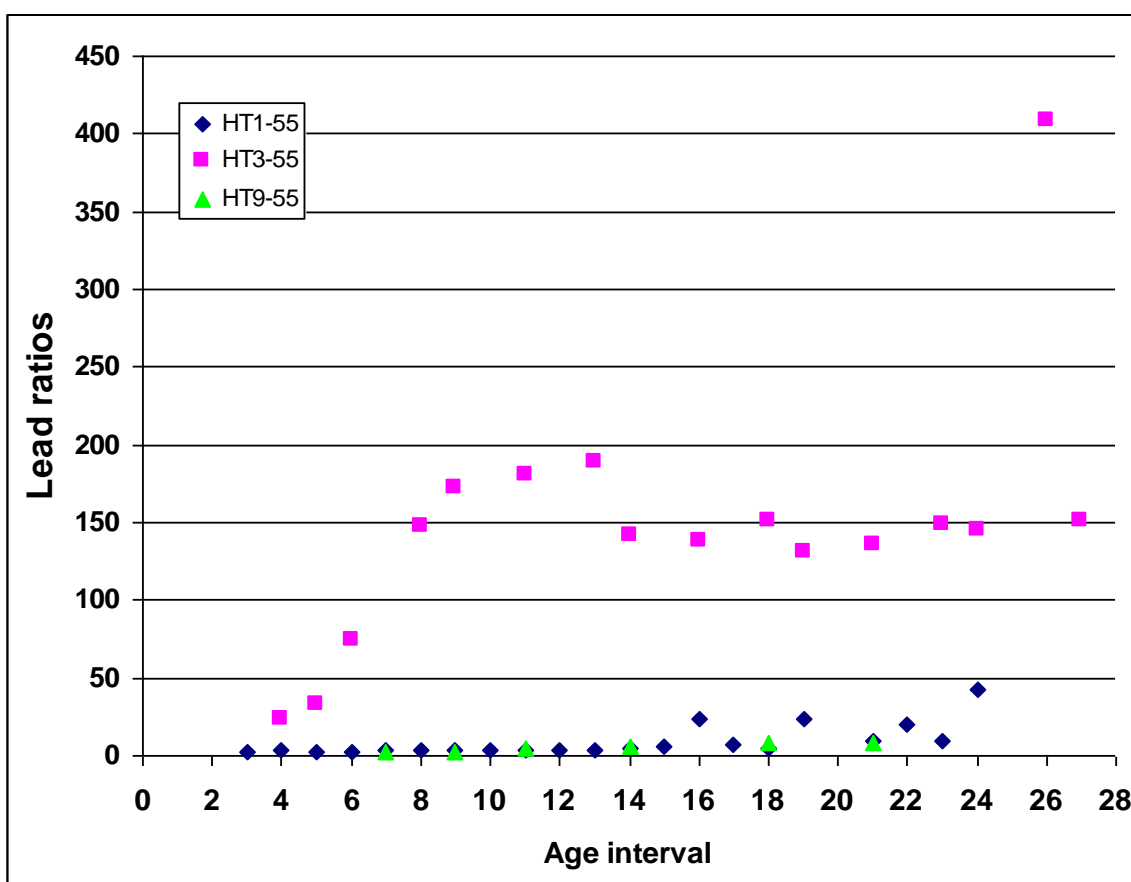
#### **Summary**

**Question 2d:** Does tooth type matter?

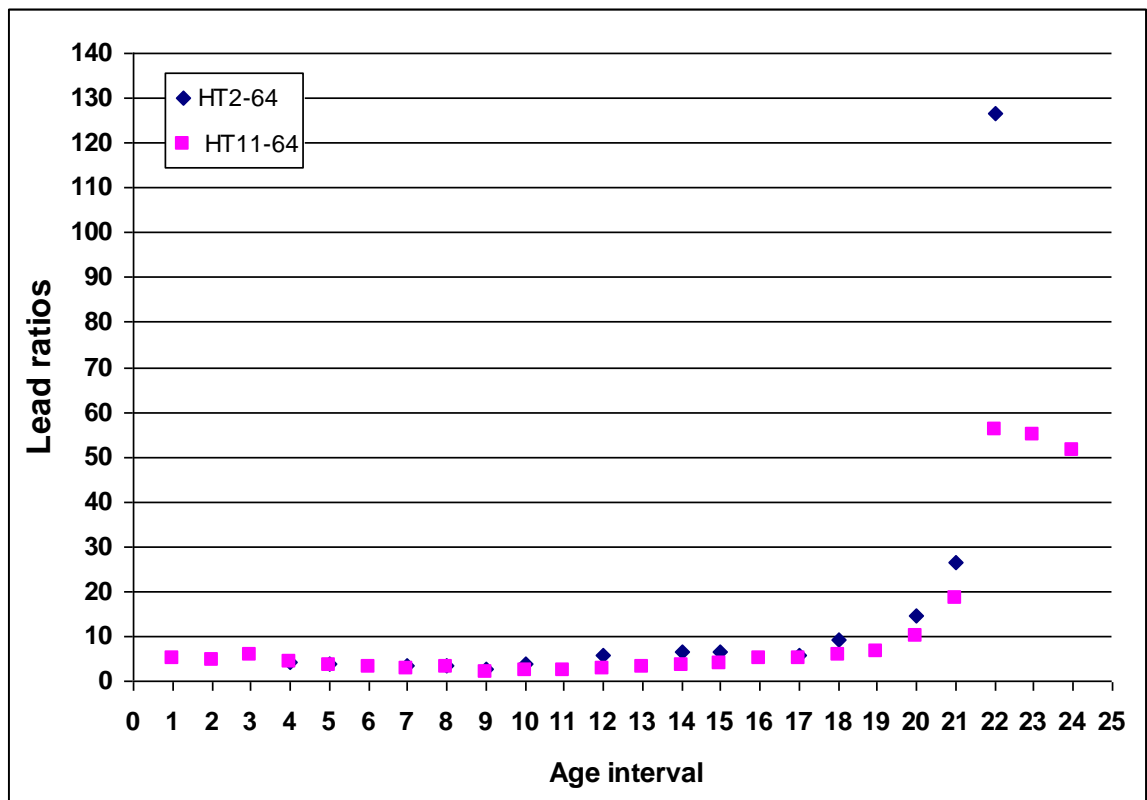
**Answer:** Tooth type appears not to be a critical in determining variations in lead ratios in the present study, although we only analysed one tooth type (molar) so recommend further study in multiple tooth types to explore potential variations of lead levels between different tooth types.



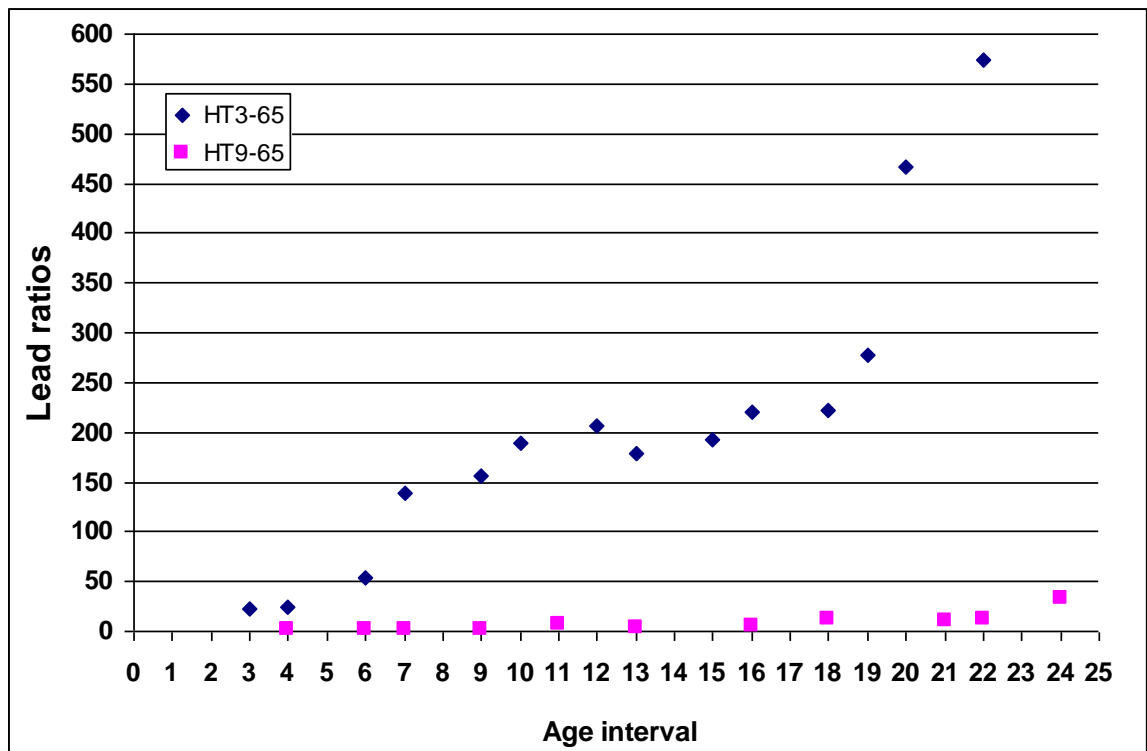
**Figure 4.29** Comparison of primary dentine lead ratios among children with tooth type 54



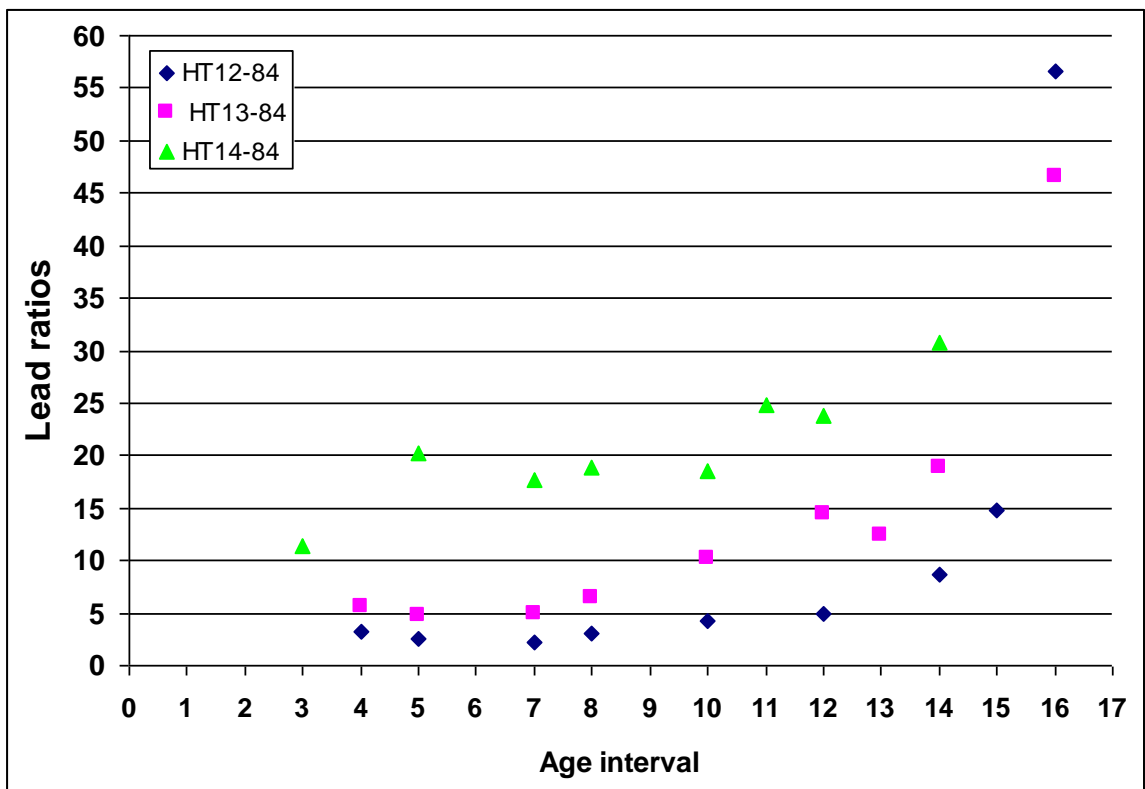
**Figure 4.30** Comparison of primary dentine lead ratios among children with tooth type 55



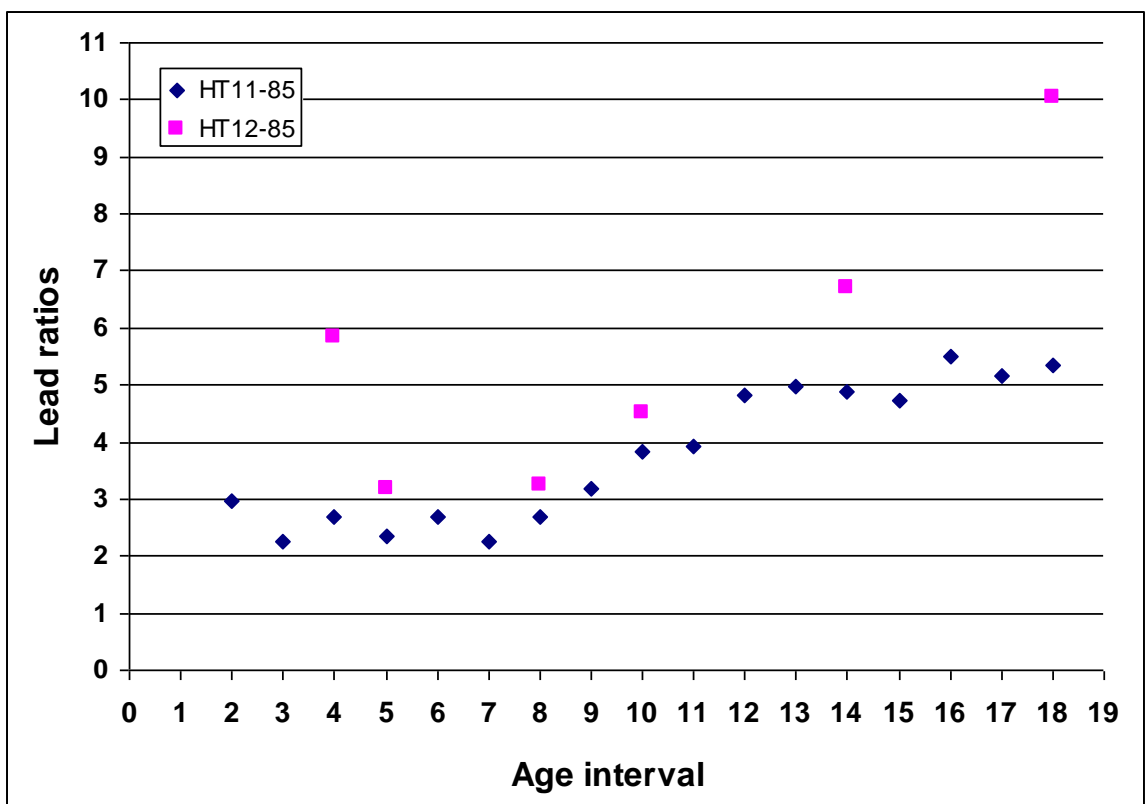
**Figure 4.31** Comparison of primary dentine lead ratios among children with tooth type 64



**Figure 4.32** Comparison of primary dentine lead ratios among children with tooth type 65



**Figure 4.33** Comparison of primary dentine lead ratios among children with tooth type 84



**Figure 4.34** Comparison of primary dentine lead ratios among children with tooth type 85

#### **4.4. Question 3: What is the relationship between tooth and blood lead levels?**

For the 15 children recruited into the Teesside sample, blood lead levels ranged from 0.52-6.83µg/dL (mean±SD=1.53±1.5), and none of the children had a BLL above the WHO threshold of 10µg/dL. The mean ranking of BLL was higher in females than in males (p=0.018). The median BLL for females of all ages was 1.41µg/dL and for males it was 0.93µg/dL. Children aged 8 years had slightly higher BLLs than those aged 6-7 years, but no significant association between BLL and age was found.

No correlation was seen between blood and tooth lead levels in the 11 children for whom tooth lead levels were assessed. The correlation between the maximum DLLs close to the pulp cavity and BLLs was not explored. Figure 4.35 shows a scatter plot of blood and enamel lead levels of children from the Teesside sample, and Figure 4.36 presents a scatter plot of blood and dentine lead levels of children from the Teesside sample.

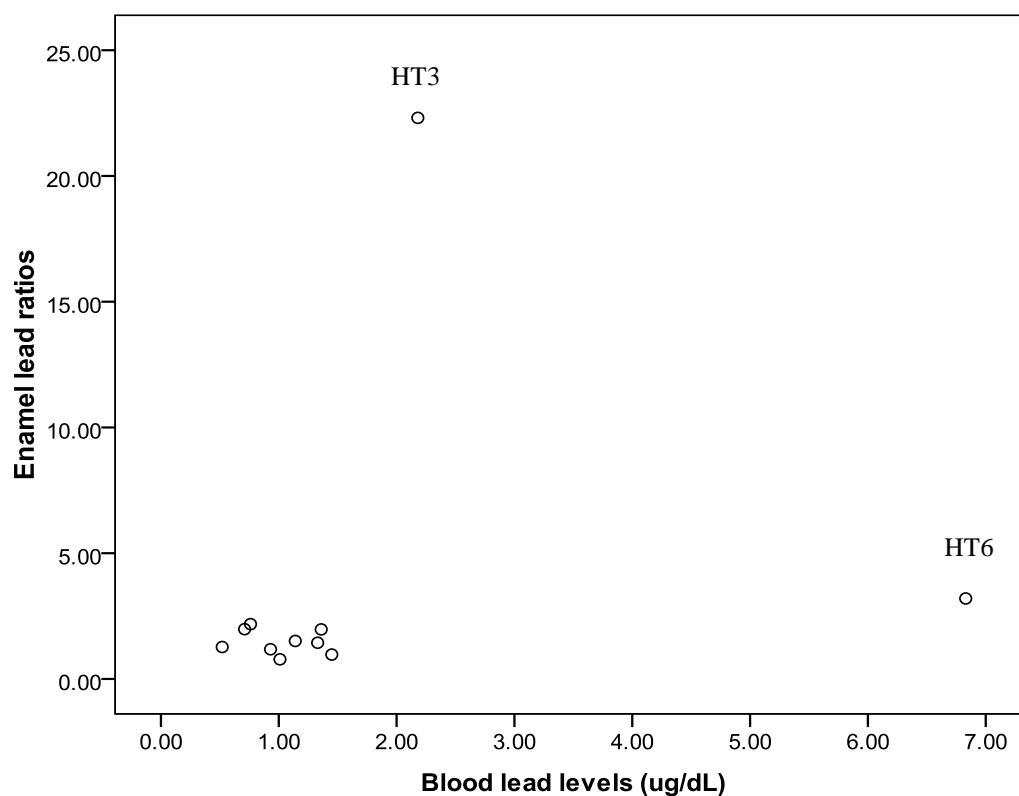
Lead ratios in enamel were consistently low (<10), with the exception of child HT3. The highest lead levels in dentine were also found in this child. Child HT6 showed the highest blood-lead concentration (6.83µg/dL), nevertheless, enamel and dentine lead ratios in this child were low.

#### **Summary**

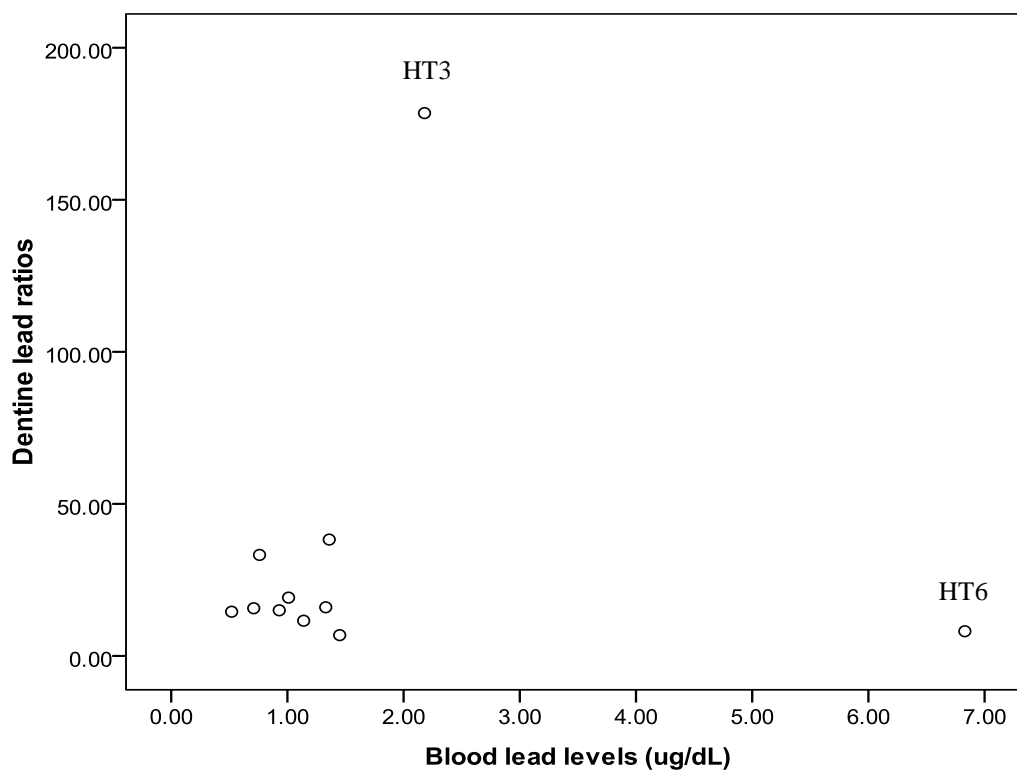
**Question 3:** What is the relationship between tooth and blood lead levels?

**Answer:** There was no statistically significant association between tooth and blood lead levels in the Teesside samples.



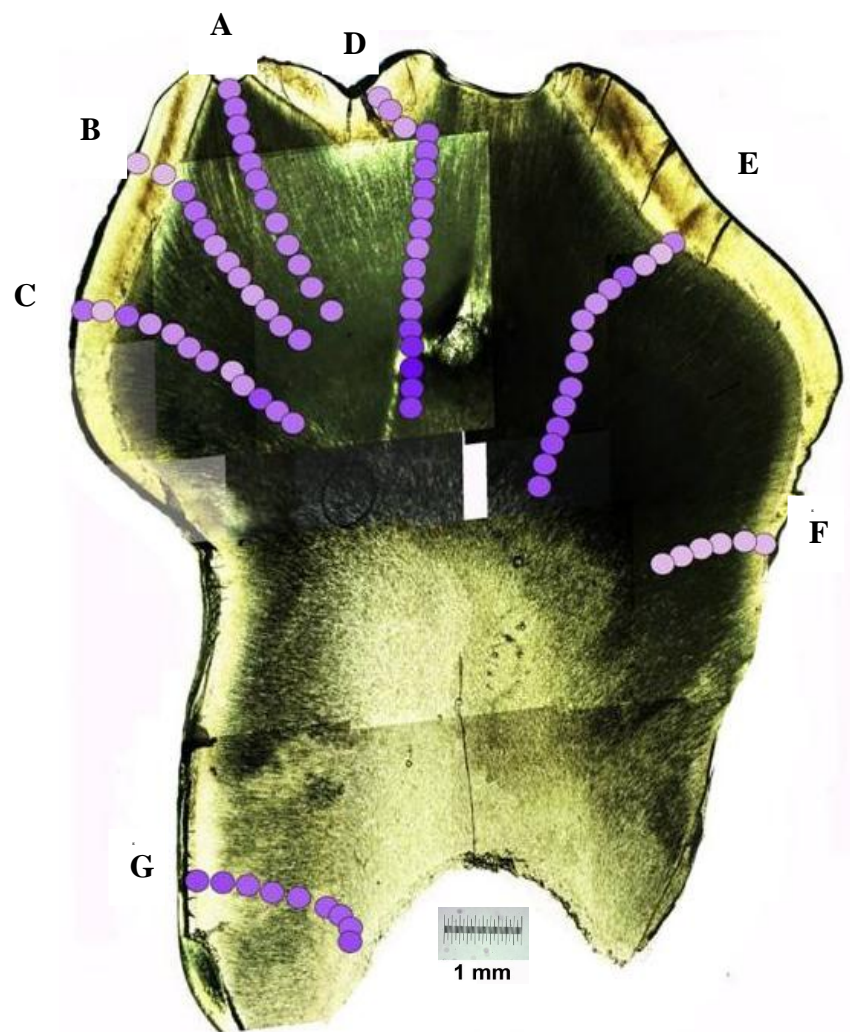


**Figure 4.35** Scatter plot of blood and enamel lead levels of children from the Teeside sample



**Figure 4.36** Scatter plot of blood and primary dentine lead levels of children from the Teeside sample

Although we expected to apply the histological technique to every child especially child HT6 who had the highest BLL of  $6.8\mu\text{g/dL}$  at 8 years old, tooth sections 65 and 74 from child HT6 were unable to undergo the histological analysis. Because of the extensive damage to these teeth from caries. The teeth could not be sectioned through the ideal plane so ages for ablation pits could not be determined. One tooth (HT6-74) was ablated. Figure 4.37 shows the spatial distribution of lead ratios in the enamel and dentine in a mandibular left first molar from this child. Seven transects (A-G) were made across the tooth surface. The pattern of lead ratio in each transect varied. Comparatively low lead ratios were observed in the enamel in transects B and D, while these ratios in transects C fluctuated between the enamel and dentine. Lead ratios in dentine for transects A-F were inconsistent. These ratios in enamel and dentine ranged from 1.43-5.96 ( $\text{mean}\pm\text{SD}=3.20\pm1.6$ ) and 1.62-73.46 ( $\text{mean}\pm\text{SD}=8.12\pm9.7$ ), respectively. Comparing the trends of the lead ratios, it can be seen from transect D that higher lead ratios were found in the dentine close to EDJ, which slightly decreased when moving to the inner dentine before increasing again close to the pulp cavity. Prenatally formed dentine in transect E showed lower lead ratios than those in postnatally formed dentine. The most striking results to emerge from the data for transects F and G are that lead ratios in these lines are relatively stable from the surface to the innermost layer. However, teeth from this child did not provide good sections due to caries, and direct comparisons with lead ratios in other teeth were difficult.



**Figure 4.37** The spatial distribution of lead ratios in a primary mandibular left first molar for HT6-74 from the child with the highest BLL

# **CHAPTER V**

## **DISCUSSION AND CONCLUSIONS**

# CHAPTER V

## DISCUSSION AND CONCLUSIONS

### 5.1 Discussion

My study was designed to explore whether milk teeth are suitable biomarkers for determining the history of exposure to lead. Such a biomarker would have the potential for helping to identify sources of exposure and highlight vulnerable stages in the life course. The key findings of the epidemiological study were that no associations were found between determinants of lead exposure and DLLs in Newcastle. The histological study showed that histological dating of growth increments in milk teeth, in combination with LA-ICP-MS analysis, could provide a timeline of exposure to lead in primary dentine from *in utero* up to three years after birth, using the true time of dentine formation and growth. After comparing two teeth from each child, and multiple transects within each tooth, I have shown that a meaningful history of exposure to lead could be obtained from a single, multi-point ablation transect on a high quality longitudinal section of an individual tooth. The histological data demonstrated that the primary dentine of deciduous teeth can be used to reconstruct a chronological record of childhood exposure to lead. The following sections discuss these findings in the light of previous research.

#### 5.1.1 Epidemiological study

The Tooth Fairy study was conducted in Newcastle upon Tyne, which is a city with a long history of lead smelting and mining in the surrounding areas, and therefore a wide range of possible environmental lead sources including soil. I analysed data on DLLs alongside data from a questionnaire both collected in 2005.

The overall mean DLL of the children in this study was low ( $0.25\mu\text{g/g}$ ). The levels from Newcastle in 2005 were lower than the mean DLLs reported by Grobler et al (2000) in primary teeth of 48 children from non-polluted areas in South Africa (Mean DLLs were  $2.23\pm 1.32\mu\text{g/g}$ ). [241] The mean DLL in the current study was also considerably lower compared to earlier studies from the UK, which had used whole teeth. Smith et al (1983) undertook a survey of 6,875 children from 168 infant schools in London between

December 1979 and April 1982, so more than twenty years earlier than the current study. They analysed a total of 2,564 teeth from 1,917 children. The upper central incisors (30.1% of all teeth collected) had a mean tooth lead level of  $5.7 \pm 2.9 \mu\text{g/g}$  (for the right incisors) and  $5.6 \pm 2.9 \mu\text{g/g}$  for the left. [325] The DLLs in the present study were also lower than those (mean $\pm$ SD of  $4.4 \pm 3.5 \mu\text{g/g}$  and  $3.3 \pm 2.5 \mu\text{g/g}$ ) reported from primary school children in Taipei and Boston (1991). [127]

In considering possible explanations for these differences in tooth lead levels, methodological differences may be responsible, as well as real differences relating to the different locations and time periods concerned. Firstly, it is likely that the decline observed in tooth lead levels over time is real and explained by control measures such as phasing out of leaded petrol and use of lead as pigment in paints. [152, 326] Secondly, a number of studies have reported that lead concentrations in teeth vary between regions, with children living in polluted areas showing higher levels than children in less polluted areas. [155, 156, 327]. Site specific factors influencing lead bioavailability, and individual factors influencing exposure, which in turn may be influenced by gene-environment interaction of lead, may also play a role in determining the different DLLs observed. [127]

Surprisingly, I did not find significant associations between established determinants of lead exposure and DLLs. None of the demographic variables such as age and sex, or socio-economic variables were statistically significant independent predictors of DLLs in this cohort.

### **Variation of DLLs by age**

Several previous studies had reported lead levels to be related to age. It has been widely accepted that young children are most at risk of lead exposure owing to their hand to mouth activities. [29, 299] The Australian Port Pirie cohort study showed variations in childhood BLLs by ages. Mean BLLs increased sharply between the ages of 6 and 15 months, and the maximum level was found at two years of age ( $21.2 \mu\text{g/dL}$ ), with decreased BLLs were found in this cohort at three and four years. [117] One possible explanation is hand to mouth behaviour in small children. In contrast, conflicting evidence is available as to whether the risk of exposure increases or decreases with increasing age in children. This may be due to different activities among the different populations of children studied and variations in patterns of exposure to environmental

lead. [328] Age was not associated with DLLs in the Tooth Fairy Study, however the ages of the children recruited were restricted to between 5-8 years old. Therefore the age group may have been too narrow to detect the influence of age on DLL.

### **Variation of DLLs by sex**

Differences in levels of lead between the sexes have been documented in some studies. In the present study, female children had slightly higher DLLs than male children (mean $\pm$ SD=0.26 $\pm$ 0.17 and 0.25 $\pm$ 0.13 $\mu$ g/g respectively); however, this difference was not statistically significant. These findings are consistent with those of Tsuji (1997), Bayo (2001) and Karahalil (2007). In contrast, Arruda-Neto et al (2009) studied lead levels in 74 teeth of children aged between 5 and 10 years old in the region of Sao Paulo, Brazil, and reported teeth lead levels in male children (1.36 $\pm$ 0.20 $\mu$ g/g) to be significantly higher than those in female children (1.14 $\pm$ 0.14 $\mu$ g/g). [299] Other studies in Egypt, Taiwan and USA have also found significant difference between lead levels and sex, with males always having higher levels. [156, 329, 330] Possible explanations may relate to different outdoor activities, areas or spaces where male and female children play, or food intake relative to body weight. [331]

### **Variation of DLLs by location and age of housing**

Many studies have reported differences in DLLs between those living in urban or rural and industrialised or non-industrialised areas. [127, 155, 156, 299, 332] The present study was designed to only include children from the Newcastle area, and so rural/urban variation was not explored. In the present study, children living in housing built before 1960 were found to have slightly higher DLLs than those living in newer housing, but again the difference was not statistically significant. Lead-based paint was used in older houses or buildings, and as a result lead particles or dust may remain in the living areas. Leaded paints pose a greater risk to young children who exhibit pica behaviour, chewing or consuming non-food items, which can result in high lead exposure. There is legislation in place to control the lead content of paints in the UK, and this hazard is decreasing as more old paints are removed or covered by low lead paints. However, it is important to note that the complete elimination of leaded paintwork in houses will take many years. [153] Children whose houses had been renovated had higher DLLs than those without renovation, with renovation potentially increasing lead dusts in the house, however, this difference was also not statistically significant.

## **Variation of DLLs by house dust**

House dust is a potential source of lead exposure. Some activities such as disruption of surfaces, window replacement, demolition and renovation generate lead dust resulting in lead accumulation in the house. Epidemiological studies have explored the relationship between human exposure and the concentration of lead in dust. [333, 334] In the present study, children living in houses with hard floors that cleaned less than weekly had the highest DLLs, although this finding was not statistically significant. The US Department of Health and Human Services recommended frequency of vacuum in the houses at least once or twice a week. [335] White and Dingle suggested that the intensive carpet cleaning reduced 50% in airborne fine particles. The reduction was done by vacuuming four times per square meter ( $\text{m}^2$ ), followed by cleaning one minute/ $\text{m}^2$  every other week. [336] The absence of an association between the frequency of cleaning and DLLs in the present study may be due to the small number of respondents with hard floors in the house (43/69 children), only seven reported floors being cleaned less than weekly. In addition, children's ages in Davies's study were younger than those in the present study and the younger age groups may have resulted in a stronger association due to age related hand to mouth behaviour. Finally, the time gap between the two studies is about 15 years, therefore lead levels in the present study are likely to be lower than in the 1990s due to the longer time since legislation was introduced to reduce lead in many products. As such lead dust may therefore not be an appropriate predictor for DLL at the current time when exposure to lead is generally low.

In the univariate analysis, I found a significant relationship between methods of cleaning floors in the houses and DLLs. Whilst only two parents reported that dry cleaning with a dust pan and brush or broom was the main method of floor cleaning in their houses, their children's DLLs were higher than the DLL of children whose houses were cleaned using other methods. My findings suggest that including methods of house cleaning should be considered when studying determinants of lead levels, although there is little previous evidence for this in the literature. For example, Leroyer et al reported that using a broom increased the amount of particulates suspended in the air. They also found that women who used a broom for housekeeping had 40% higher average BLLs than those who did not use a broom. [337] Lead containing dust may accumulate on carpets, floors, and other surface areas. If inappropriate cleaning methods are used, dust may not be eliminated, resulting in an increased chance of exposure to lead dust by inhalation. Furthermore, lead containing dust on walls may fall to the floor where young children are likely to crawl, or



dust may adhere to children's toys. Children's hands may thus become contaminated with lead dust, and ingestion is then probable by hand to mouth contact. [131, 338]

Special cleaning methods are recommended to decrease lead dust loading, such as vacuum cleaning or detergents, and wet cleaning with a mop. [339] The USA Department of Housing and Urban Development (HUD) guidelines for the control and removal of lead dust loading on floors, walls, ceilings, and other horizontal surfaces recommends a three-step cleaning process: vacuuming with a machine equipped with a high-efficiency particulate air (HEPA) filter; wet washing with appropriate detergent; and vacuuming with a HEPA-filter vacuum. Using these steps, a maximum of 80% more dust lead loading was removed compared to simple cleaning processes. [338]

### **Variation of DLLs by hand to mouth behaviour**

Hand to mouth behaviour is also a potential pathway for the uptake of lead in children living in areas with high soil lead levels. Results from the Newcastle Byker incinerator study showed elevated lead levels in soil, mainly in areas away from the incinerator due to historic industrial activity. [151] Contaminated soil in playground areas, gardens, and land around housing and recreation areas where children spend time means that such areas are potential sources of exposure to lead. In addition, smaller sizes of soil or dust particles can lead to a higher lead body burden. For example tiny particles with diameter less than  $0.25\mu\text{m}$  emitted from petrol are well absorbed by inhalation, whereas larger particles from lead on paint chips or dusts are less easily absorbed, and are less bioavailable. [52, 340, 341] When measuring the lead content in samples of house dust categorized by particle size in Cincinnati, Ohio, in the USA, it was found that lead dust concentration was independent of particle size, and that the bulk of the dust particles were concentrated in the smaller particle size ranges. Almost 77% of lead was present in particles with diameter less than  $149\mu\text{m}$ . This distribution of lead in small particles would maximize intestinal absorption. [342] In the present study, only one child was reported to eat soil and dirt (pica behaviour) and their DLL was  $0.4\mu\text{g/g}$  which is higher than mean DLL of  $0.25\mu\text{g/g}$ .

### **Variation of DLLs by lead piping**

Lead water pipes can be a source of lead exposure, particularly in old houses which have not had pipe work replaced. [131, 159] Children living in houses which previously had

lead water pipes had higher DLLs than other groups; however, this was not statistically significant. This may be due to the DLLs being measured after the lead pipes had already been replaced.

Related to lead piping as a potential source of exposure, the amount of tap water drunk by children was a statistically significant independent determinant of higher DLLs. However, contrary to expectations, it was the children who drank least tap water who had the higher DLLs. It was not clear how precisely the amount of tap water drunk by the child was estimated by parents, as such the routes by which a child might have been exposed to lead via tap water or other sources such as breast milk still need to be clearly explored. Children may be exposed to lead via both pathways. In the present study, DLLs in children who had ever received bottle milk-feeding were slightly higher than those who never received bottle-fed milk, which might be expected if the water used to make up the formula was contaminated by lead, but this finding was not significant. Lead contamination of human milk may also increase exposure in breast fed infants whose mothers have a high body burden of lead [343], however the present study did not show a significant link between breast or formula milk and lead contamination.

### **Variation of DLLs by socio-economic status**

There is evidence for a link between social inequality and health risks including lead exposure. [123, 344, 345] The US Centers for Disease Control and Prevention, CDC (2005) identified low income as one of the most important determinants of lead poisoning. A considerable amount of research has been published showing a strong relationship between high lead levels and low income. [346] Many studies from the USA have reported that children from poor families are more likely to be vulnerable to environmental heavy metal exposure with associations being observed between DLLs and low income, education, and nutrition. [152] American children living in older houses and areas of deprivation were at greater risk of having elevated BLLs, suggesting that older, lesser-valued homes were more likely to have deteriorated-leaded paint. [347]

In the present study, socio-economic status was assessed using the educational level of parents, household income, and deprivation as identified by Index of Multiple Deprivation scores (IMD Scores) of the child's residential or school areas. The IMD scores in the UK parallel the census-tract level indices of area deprivation for the USA.

[348] In contrast to previous studies, I did not find a significant association between socio-economic status and DLLs.

Children whose homes were in moderately deprived areas (IMD scores 20.01-30.00) had higher DLLs than those whose homes were in the most and least deprived areas, however this difference was not statistically significant. Most children were recruited from schools in the least and most deprived areas and no significant difference between DLLs and school area IMD was found. A previous study in Saudi Arabia reported that a possible source of high lead exposure was school location, especially in highly contaminated areas. One-fourth of children aged 6-12 years attending schools in urban areas with high traffic density had an average BLL  $\geq 10\mu\text{g/dL}$  because there was heavy vehicle emission in the areas. [349] Similar trends of having higher lead levels in high exposure areas have been found in other investigations. [33]

Most parents (60%) in the present study were well educated and the highest mean DLLs were found in children whose parents had the highest educational levels, although their DLL was similar to the DLLs of children whose parents had only attended secondary school.

A study undertaken by CDC in 1997 reported that while BLLs in American children decreased as they grew older (between 12 and 72 months of age), children of low income families still had higher BLLs than more affluent children. [135] In the UK, the Health Protection Agency (HPA) has recognised that exposure to chemicals and poisons in the environment is greater in poor and disadvantaged areas. Furthermore the HPA acknowledged that children are especially affected by health inequalities, poverty and being in vulnerable groups such as certain ethnic minorities. Often no beneficial effects in terms of cognition, behaviour or neurophysical function can be gained from treatment for exposure to lead such as chelating therapy in preschool children. [350] Consequently evidence of exposure and the identification of susceptible groups becomes the public health target. In the present study, no significant relationships were found between socio-economic status and high DLLs. This lack of association may be because most children were recruited from moderate deprivation areas, their parents were well-educated, and had average monthly incomes.

Related to socio-economic status, poor nutrition in children may increase their susceptibility to lead exposure. For example, iron deficiency increases the rate of uptake of both iron and lead from the gastrointestinal tract. As a consequence iron deficiency often occurs in conjunction with lead exposure. [351] In this study population only one child had been diagnosed with iron deficiency, and had a DLL of 0.11 µg/g.

### **Variation of DLLs by occupation**

Children living in houses where household members reported taking part in occupational activities with potential for lead exposure had slightly higher DLLs than children from households not reporting these occupational activities, however this difference was not significant. One possible explanation for this may be the small number of parents involved in work with possible exposure to lead (n=43). In addition, the study relied on the self-reports of parents who may have had little knowledge of the potential for lead contamination in their workplaces. Furthermore, parents or household members may have reported activities associated with lead, but no actual tests of lead at work were undertaken. Finally, parents were not asked about their hygiene practices in the workplace, which could affect the likelihood of them bringing lead dust home on work clothing or uniforms from which it could have contaminated the house leading to the potential for childhood exposure. In failing to find an association with occupation the results from the Tooth Fairy study differ from those of previous studies which showed lead levels related to occupational activities. [175, 337] On the other hand current findings are consistent with those of other studies, especially those in areas where lead petrol has been phased out. [352]

It should also be noted that BLLs have fallen in workers for every sector across UK industries over the period 1995-2007. BLLs above the current UK suspension limit of 60 mg/dL fell from 4.8% in 1995 to 0.6% in 2007 suggesting that parental occupation as a source of exposure is now less likely than previously. [353]

Although no significant effect of occupation was found, the spread of DLLs within each child (shown in Figure 4.5) showed that one child with DLLs > 0.5 µg/g (an arbitrary cut off) and a statistically greater spread of DLLs had parent who were involved or worked with electronics. Occupational exposure therefore remains a plausible risk factor for exposure even in the current study.

## Variation of DLLs by other factors

Parents of the Tooth Fairy study children were asked about food storage in pottery with leaded-glaze, lead crystal or pewter and lead soldered cans. Only one parent reported the use of lead-glazed pottery, but about 30% of respondents did not know whether their food containers included lead components. No significant effect of this variable on DLLs was found which may be due to parents' uncertainty about this source of exposure.

Smoking has been related to lead levels in humans, [169, 354-356] other studies have found no such relationship. [357, 358] Children may be exposed to lead by inhaling second hand tobacco smoke. In addition, households may be contaminated with lead dust absorbed from smoking indoors. [359] In the present study, a higher mean DLL was found in children whose household members smoked compared to those who did not, but this difference was not statistically significant. A possible explanation may be the limited reliability of parental reports of smoking. There was no specific evidence of underreporting. Furthermore there were only thirteen children whose parents were smoking in the house during the study period. In addition, the use of a dichotomous (yes/no) variables prevented the estimation of the quantity of cigarettes smoking daily.

The number of people living in the household was explored as a determinant of DLLs. Children who lived with a single adult had higher DLLs than those who resided with at least two adults, but the difference was not statistically significant. In addition, children who lived with at least two siblings aged 5 and over had higher lead levels than those who lived with only one child, although again the difference was not significant. Number of people live in the house and lead exposure is unclear. The possible risk may be from parents who involved with lead and bring potential risk from cloths or relevant exposure. Take-home lead exposures may involve when parents and/or people whose jobs expose them to lead wear their work clothes home or wash them with the family laundry. It may result when they bring scrap or waste material home from work. [345]

Parents were asked in the present study about the use of traditional Asian kohl eyeliner. Only one parent responded affirmatively and her child had a mean DLL of  $0.27\mu\text{g/g}$  which was higher than mean DLLs of all children ( $0.25\mu\text{g/g}$ ). Asian cosmetics contaminated with lead are a known source of lead exposure. [38, 177, 178] Previous study in Israel found that BLLs were significantly higher in infants aged 6-16 months whose mother reported using kohl, and the authors concluded an association between

applications of kohl to the infants or mother's eyes and elevated BLLs in infants. [178] Traditional Asian kohl may be a risk factor of lead exposure especially in the people who normally use this product.

Several studies have focused on soil as a major factor in determining child exposure to lead. [145-148] Located on the river Tyne, Newcastle was previously a highly industrial city. Evidence of heavy metals including lead contamination has been found in soils and allotments. [149] When particular concerns were raised about heavy metal concentrations in stack emission from a municipal solid waste incinerator in Byker, the City Council of Newcastle upon Tyne conducted a study of the pollutant contamination of soil. [360, 361] Only a few children from the Tooth Fairy study were recruited from the Byker area, and DLLs for those children were slightly higher than those children living outside the Byker areas but this difference was not significant.

In summary, it was surprising that no significant relationships were found in the present study between known determinants of lead exposure and DLLs in this cohort from Newcastle, although it was not possible to explore soil lead exposure. These findings contrast with those of studies undertaken in other parts of the world. Determinants such as the age of housing and socio-economic status are often reported as important determinants in studies of blood lead in the USA but were not found to be relevant in this setting. In the light of the current evidence on the toxicity of lead to children, further work to characterise sources of lead exposure in the UK is required.

Given the lack of association with previously established determinants of lead exposure it was difficult to conclude from the Tooth Fairy Study whether or not dentine is a suitable biomarker for identifying determinants of lead exposure. Therefore it became necessary to study the methodology used in greater detail, leading to the development of the new histological technique, which would allow us to assign time intervals to specific sections of the teeth and to study the full history of lead exposure.

## **5.1.2 Histological study**

### **Can our histological technique recreate the history of lead exposure?**

The main question I was trying to address with the second part of my research is whether or not teeth can be used as a biomarker for the history of lead exposure. The following sections discuss my results based on the research questions asked in the present study.

The use of spatial lead distribution in enamel and dentine across the surface of the tooth in investigating the history of exposure has the advantage of being able to identify exposure during an individual's early life. This depends on the hypothesis that lead levels at specific anatomical points across tooth surfaces reflect the intensity of exposure at a particular time in an individual's life. Similar ideas about the distribution of lead levels in enamel have previously been proposed by Ericson (2001), Dolphin (2005) and Humphrey (2008a) [245, 288, 289], but only a few studies of different areas of dentine have been conducted. [13] Among these studies, only a limited number have considered different parts of the tooth in mapping long term lead exposure, and as yet no consensus has been reached on an acceptable method to determine ages of lead exposure in different parts of the tooth. The development of a histological technique to identify tooth lead levels has therefore been conducted in this study.

#### **A: Should we sample enamel and/or dentine?**

Previous studies have reported the use of lead in teeth as a biomarker of the past exposure of children. Over the past three decades, these studies have most often analyzed whole teeth, which were digested in acids. [237, 302] Because we know that different components of human teeth incorporate lead differentially, analyses of whole teeth do not allow the time-specific distribution of this metal to be assessed. [286] There is therefore increased interest in studying lead exposure in different parts of the tooth to account for this differential incorporation over time. [13, 286] A recent study of lead in the deciduous teeth suggested that circumpulpal dentine was the most appropriate tissue in which to sample cumulative lead exposure [241]; however, there is as yet no consensus on an accepted measure of lead from different parts of the human tooth.

For the teeth collected from the Teesside area in Northeast England, dentine had higher lead levels than enamel, and within the dentine there were higher lead ratios in the postnatal versus prenatal dentine. The highest lead ratios were consistently found in dentine near the pulp cavity. The findings of the present study are consistent with those of Arora et al (2006) which showed that lead levels in dentine are significantly higher than those in enamel. Several other studies in the past have reported similar results. [13, 241, 286] Using histological techniques to identify age specific exposure to lead, I showed that dentine can be assessed from 5 months before birth to up to 3 years after birth, a longer period than reflected in enamel (5 months before birth to 8 months after birth). Dentine continues to form after the enamel of the tooth crown has formed, and the periods of dentine development *in utero* until root completion are longer than those in enamel implying that the duration of exposure should be better assessed using dentine. [239, 244]

The results from the epidemiological study showed that lead levels in enamel were lower than those in dentine which is in agreement with the findings of the histological study. In some children, enamel lead levels were not detectable. Enamel incorporates such low levels of lead that it may be difficult to use it in identifying history of lead exposure, especially in low exposure populations, although it works well with other trace elements like strontium based on a study of Humphrey. [245] In addition, in carious teeth, the dentine may be protected by the enamel with less structural change in the hydroxyapatite matrix (although see discussion of Child 14 below). Although I did not find an association between DLLs and determinants of lead exposure in the epidemiological study, dentine shows higher lead levels than enamel, and mineralise through early life. We therefore focused on dentine in the present study although we continued to begin our ablation transects in enamel.

## **B: How many transects per tooth are required?**

The findings in the second phase of the present study showed that consistent ratios at the same age interval were observed in primary dentine. Focusing on the initial four teeth with multiple transects, no systematic difference was found between lead ratios at the same age interval. Every transect in the initial four teeth showed the same pattern, with lead ratios relatively flat except in the dentine close to the pulp cavity, i.e. they showed no dramatic changes in lead levels with age. We observed relatively consistent ratios between teeth from the same child, and at the same age within each tooth, indicating that



in a good quality section the history of lead exposure may be determined using one ablation transect in primary dentine. In addition, the age at each ablation point can be measured and lead ratios mapped across the tooth transect at a higher temporal resolution than a recent study by Arora et al (2006), who reported clear differences in enamel and dentine lead concentrations during the pre- and neonatal period. [13]

After dentine and enamel have differentiated prenatally, dentine continues to be laid down by odontoblast cells that line the pulp cavity. These processes act as biological pathways in transferring and controlling the movement of mineral ions from the blood plasma to the sites of dentine secretion. [362] In every transect for the initial four teeth, lower lead ratios were found in the first layer of mineralised dentine close to the EDJ, known as mantle dentine which is the outer most layer of crown dentine. This less mineralised layer consequently showed the lowest lead concentration. After that, the growing of the crystals in reducing collagenous materials results in the area of primary dentine where lead ratios slightly increase with age from the EDJ towards the pulp cavity with a rapid increase found especially in the primary dentine close to secondary dentine, and the highest lead ratios in the dentine close to the pulp cavity. Based on the trend observed in every transect, it seems that the dentine has mineralised in a consistent manner from the EDJ to the pulp cavity. The pattern of lead ratio distribution in primary dentine at the same age in the present study differs from trace element distribution in enamel due to different mineralisation. Once dentine has been completely mineralised, it does not incorporate lead differentially the way that enamel does. These results suggest that consistent lead ratios are found at the same age and may be explained by the consistency of dentine mineralisation across the tooth crown.

As described in detail before, the present study modified the methods developed by Humphrey and colleagues [245, 246, 284, 324] to identify lead ratios at specific ages focusing on dentine. In enamel, Humphrey and colleagues (2008) demonstrated that calcium normalised strontium intensities at the same age varied depending upon where samples were located in the crown of the tooth. [245] This is due to the gradients of enamel mineralisation across the tooth crown. [245, 324] They also determined that a reliable chronology could be determined when controlling for distance from the surface of the tooth. [324] Dentine and enamel mineralise differently but dentine also continues to mineralise after its initial formation. Because of the inconsistencies in trace elements in enamel at the same age, we decided to explore several transects across the tooth surface in

order to investigate the pattern of lead mineralisation in dentine. As stated above, consistent lead ratios at the same age were found in every transect of individual tooth samples.

One ablated transect in a good quality section may be sufficient to determine history of lead exposure because age and lead ratio can be identified at each ablation pit. The findings also strongly support the assertion that each laser ablation pit provides a real time interval based on the number of days in pre- and postnatal periods. No systematic difference between lead ratios at the same age interval was found in this small sample. As such, I believe this technique can therefore capture the history of exposure from five months in utero to three years of age using one transect per tooth. The combination of histological dating of growth areas of milk teeth in combination with LA-ICP-MS analysis is likely to be useful in establishing the history of lead exposure in children using only one transect.

The results of this study indicate that the highest lead ratios are found in postnatal dentine near the pulp cavity. These results are consistent with those of other studies. [13, 241] There are several possible explanations for this. Due to the presence of dentinal tubules, dentine is highly permeable. The number of tubules in dentine varies depending on where in the tooth it is found. [363] The number of tubules per unit at the pulp cavity is almost 4-5 times more than at the outer surface of the dentine. In addition, the ratio of number of tubules between the outer and inner surface of dentine is about 5:1. Therefore, these tubules are farther apart in younger dentine and pack closer together near the pulp cavity. [255] So the permeability of dentine increases from the EDJ towards the pulp. It is possible that high lead ratios in the area near the pulp cavity are due to this permeability.

Reviews of studies of common trace elements reveal that the patterns of lead distribution in dentine are the same as that of fluorine, zinc, strontium, and manganese in other studies. [244, 296, 297] Furthermore, during the past 30 years, evidence increasingly suggests that lead levels in root dentine are higher than those in crown dentine. However, no comparison of lead concentrations between crown and root dentine was conducted in the present study.

Primary dentine lead ratios in two teeth from child HT3 showed a different pattern suggesting that lead exposure in this child varied over time. This child had the second

higher BLL (2.18µg/dL). In this child, the ratios of lead in the primary dentine were low from birth until 1 year of age. The ratios then increased markedly between 1-2 years of age after which lead ratios gradually decreased until the end of age interval observation. Based on this finding, we conclude that primary dentine can identify changes in lead exposure over the early life, and that in good quality sections only one transect per tooth is required to construct history of lead exposure.

Since consistent lead ratios at the same age interval were found in all transects from initial four teeth, one ablation transect was then applied to the 15 remaining teeth in the third phase of the study. Intra- and inter-individual variability in lead levels in teeth from the same and different children were also investigated.

### **C. How many teeth per child are required?**

To assess intra individual variability, I collected two teeth from each child. The findings show that consistent lead ratios at the same age are found between two teeth from the same child with the exception of child HT14 whose tooth 84 had 4-5 times higher lead ratios than those found in tooth 74 at the same age interval. One possible explanation for this significant difference lead ratios between two teeth for this child may be tooth caries. For tooth 84 with no roots, tooth caries may have resulted in demineralisation of the underlying apatite particularly the inner dentine resulting in inconsistent lead ratios. This is in agreement of Zavgorodniy et al (2008) whose findings suggested that there are untrastructural changes within different zones of carious dentine compared to sound dentine sections. [364] However, for a child such as HT3 who may have had higher environmental lead exposure the two teeth from this child also showed consistent lead ratios at the same age interval.

Between-tooth differences were found to be small and reproducible differences in exposure to lead can be quantified using a single, well positioned, laser ablation transect in the primary dentine in a longitudinal section of a single sound tooth. However, the current study is limited by a small sample size and an expansion of this study with a larger sample size in high-exposed samples and/or areas, and the collection of multiple teeth from each participant would allow further confirmation of the reproducibility of the method developed here.

Most of the remaining teeth showed low lead ratios in primary dentine. When lead ratios for each transect between two teeth for each child were compared, lead ratios in primary dentine were consistent and showed the same pattern of lead distribution across the tooth surface, except child HT3. Primary dentine lead ratios in two teeth from child HT3 showed a significant pattern, identifying history of lead exposure. The two teeth showed similar trend for this child, of a low lead ratio in primary dentine from birth until one year of age, ratios then increased markedly between 1-2 years of age, after which time the gradually decreased until the end of age interval observation. Although I did not collect data of past history of lead exposure for child HT3, a possible explanation for lead exposure in younger children is hand to mouth behaviour. If child HT3 had hand-to-mouth contact with contaminated lead objects or ate substances not normally eaten such as soil and paint chips, high lead concentrations would then be possible. The primary dentine lead levels peaked in this child at around two years of age, which agree with the findings of other studies. [10] Focusing on child HT14, higher lead ratios was found until one year after birth. Hand to mouth behaviour may also be a possible explanation for these high lead ratios. However, further research is needed. The trend of exposure found in child HT3 indicates that our technique can identify varying exposure at the different times in a child's life. If a peak of exposure occurs during the child's early life time, our method can capture this exposure as a dramatic rise in lead ratios. If the exposure to lead was consistently low, as in the majority of children from this sample, this exposure history can also be observed using our technique.

Our techniques can show the history of lead exposure at different stages of tooth development throughout the early life, giving a far more detailed picture of exposure than BLLs. With exposure able to be assessed in time intervals of ~42 days our technique provides an exciting insight into patterns of pre- and post-natal lead exposure. As previously showed by Arora and colleagues (2006), using the neonatal line as a fixed point allows us to distinguish between plasma lead levels corresponding to the mother's blood lead during pregnancy and the child's ambient exposure to lead after birth. A significant change was seen in child HT3 where dentine lead ratios were low between birth until one year after birth, then the ratios significantly increased between one and two years after birth. Although primary dentine lead ratios were low, the ability to detect measurable differences indicates the potential of LA-ICP-MS to provide information on lead exposure during early life development. Based on this finding; primary dentine can

provide informative results regarding lead exposure and may be a good area of the tooth surface to sample in order to identify long term lead exposure.

In summary, the findings from a small set of good quality sections showed differences in lead ratios between the two teeth from each child were small, and we are not providing sufficient evidence that one tooth is sufficient. We then recommend an expansion of this study with a larger samples and the collection of multiple teeth from each of the participants would allow refinement of the histological technique proposed in the present study.

#### **D. Does tooth type matter?**

The findings of the present study show that most of the children had consistent primary dentine lead ratios at the same age, irrespective of tooth type. Lead ratios were less consistent in dentine closest to the pulp cavity. For child HT2 (teeth 54 and 64) and HT14 (teeth 74 and 84), variation in the lead ratios in primary dentine at the same age interval were seen. In child HT2, teeth 54 and 64 are upper first deciduous molars located on the right and left sides of the deciduous tooth dentition respectively, while teeth 74 and 84 for child HT14 were the lower left and right deciduous molars. These teeth usually have equivalent structures, periods of development and mineralisation patterns. So it is difficult to explain the differences in primary dentine lead levels at the same age found in these two teeth, but it may be related to the oblique nature of these sections. It was found that transects in both teeth were not sampled along the dentine tubules potentially resulting an error of the measurement.

Previous studies suggest that tooth type may influence lead levels, especially in circumpulpal dentine [365] Tooth type may influence lead levels because different teeth form and erupt at different times in the human's life, and so their lead exposures histories may not be the same. [237] So, it is crucial to specify the type of tooth used for analysis, and if possible, whether it comes from the upper or lower jaw. In deciduous teeth, the age of eruption is in the order incisors>canines>molars. [239, 243]

Tooth type is a known factor in variations of lead concentrations, for example in a comparison of dentine lead levels in deciduous teeth from two cohorts in Taiwan and Boston, the central incisors had higher average lead levels than those in lateral incisors. [127] The inter individual comparison of lead ratios in primary dentine between children

whose teeth were the same tooth types (tooth types 55, 65, 84 and 85) showed large variations in lead ratios for tooth type 84 from three children. One possible explanation may be that these teeth represented different periods in different children. For example in child HT12, the ablated pits covered the time period 54 days (~2 months) before birth to 575 days (1 year 7 months) after birth, for child HT13, 34 days (1 month) before birth to 514 days (1 year 5 month) after birth, and for child HT14, 86 days (~3 months) before birth to 430 days (1 year 2 months) after birth. Another possible explanation for a difference in lead ratios may be differences in individual exposures. [127]

Deciduous incisors and molars were collected in the epidemiological and histological studies respectively. Lead levels in dentine between these cohorts were different, which may be due to calcification differences between incisors and molars. Different tooth types undergo calcification at different times. For instance, central incisors initiate calcification at 3-4 foetal months and the coronal dentine is mainly completed by the third month after birth. Deciduous molars initially calcify *in utero* and the process continues progressively during the first year after birth. If the incorporation of lead occurs during this calcification, variations in lead levels due to different time spans of calcification would be expected. [127, 244] Based on the fact that the upper central incisors are the oldest primary teeth, they should be sampled if the aim is to identify prenatal lead exposure. Nevertheless, the results from the epidemiological study which sampled dentine in incisors did not find significant associations with likely determinants of lead exposure, although the chronology of lead exposure during early life was not able to be explored in this sample.

In summary, based on our results on deciduous molars it seems likely that one tooth does work; however a larger sample is required to confirm this.

## **F: What is the relationship between tooth and blood lead levels?**

BLLs ranged from 0.5 to 6.8mg/dL, and none of the children had a BLL above the WHO threshold of 10mg/dL. The median BLL for females was 1.45µg/dL and for males 0.9µg/dL. These values were lower than those in a survey of BLL as part of the 1995 health survey of England, where the median BLLs in children over 6 years old were 1.7µg/dL and 2.3 µg/dL for females and males respectively. [366, 367] This finding is

consistent with trends in BLLs in children which have substantially declined over time due to the fact that the UK has cut lead emissions by 97% since the 1970s. [366]

Child HT6 in had the highest BLL of 6.83mg/dL, however lead levels in the enamel and dentine of this child are within the range of the other children in this study. It is necessary to note that these high BLLs represent only very recent exposure, so are not easily compared with the DLLs. There were no available data on age-related exposure using tooth lead levels from this child due to tooth caries.

Child HT3 had the second highest BLL and also the highest dentine lead concentrations. Previous studies have reported a relationship between BLLs and tooth lead levels. A study in Poland of lead in whole deciduous teeth and capillary blood of 6-year-old preschool children found a positive relationship between these two markers. [368] Other studies have found a positive correlation between lead levels in blood and those in either enamel or dentine. [199, 369] I did not observe any association between BLLs and DLLs or ELLs in the present study, although this was a small sample. Although there was no association between BLLs and tooth lead levels in the present study, the findings demonstrated that teeth might be a better biomarker for considering health outcomes of lead because chronological records of history of exposure can be constructed using tooth lead levels.

### **5.1.3 Overall discussion**

Previous studies have used BLLs to identify lead exposure in humans, but this measure reflects only recent exposure due to the short half-life of lead in blood. [5] For more than three decades, teeth have been used to assess concentrations of trace elements using whole teeth, or components of enamel and dentine, however this approach frequently destroys the tooth structure making it impossible to measure time-based element distribution using the chronological period of tooth development. [240, 286] More recently research has assessed deposited metal levels in enamel and used this matrix to study past metal exposure. [288, 289] More recent evidence and these findings from my PhD suggest that dentine can be used to examine past history of exposure because it incorporates lead as it mineralises throughout early life and records age specific rather than time averaged exposure.

The epidemiological study was designed to identify the determinants of lead exposure using DLLs. I found that lead levels in dentine tend to be higher than enamel, and DLLs represents longer time periods of exposure than enamel. However, statistically significant associations between known risk factors and lead levels in dentine were not found. The epidemiological study suggested that the sampling technique in DLL may not have been sufficient.

Human teeth represent a chronological record of exposure to trace elements during an individual's development from conception to tooth exfoliation or extraction. Different parts of teeth have been studied for lead exposure and levels of lead have been found to vary. Because different components of human teeth incorporate lead differently, the assessment of lead as a measure of exposure in chronologically specific parts of teeth has attracted attention, and the incremental growth lines observed in enamel and dentine in primary teeth offer an opportunity to use dental lead levels as a biomarker of the history of lead exposure. In particular, the findings of the histological study indicate that lead ratios in primary dentine are consistent between teeth from the same child, and at the same age within each tooth, indicating that it may be possible to determine the history of exposure using one ablation transect in the primary dentine.

Overall, the results of this study provide useful information which demonstrates the value of analysing different areas of the structure of the tooth rather than the whole tooth. The histological technique developed in this study provides evidence that measuring incremental growth lines on ground sections reflects the rate of dentine formation and age at mineralisation enabling life time lead exposure to be identified. The combination of the histological dating of growth areas of milk teeth in combination with LA-ICP-MS analysis is likely to be useful in establishing the history of lead exposure in children.



## **5.1.4 Strengths and limitations of the epidemiological study**

### **Strengths**

1. The Tooth Fairy Study was the first study in the North East of England to focus on the environmental and socio-economic determinants of lead exposure using dentine lead levels.
2. The study is one of the largest studies to explore lead levels using dentine and enamel.
3. The results triggered us to question the value of ablation of pre- and postnatal tissue for assessing lead levels and consequently we developed the histological technique to confirm the value of this biomarker.
4. Although no significant association was found between known determinants of lead exposure and DLLs; some factors, such as methods of cleaning hard floors in the house, amount of tap water that the child drank daily and growing fruits or vegetable in the garden were significantly associated with DLLs in univariate analyses.

### **Limitations**

1. No measurements were taken of lead in air, soil, and dust despite these being potentially important sources of exposure. Lead is relatively immobile in soils and can therefore be a long-term source of lead exposure. [146, 370] Two studies of soil lead levels in the Newcastle area have been undertaken. [360, 361] The mean lead concentration in allotments at Walker Road was 975mg/kg, and in allotments with ash related to the Byker incinerator was 484mg/kg. [361] These studies reveal that soil lead levels in some areas in Newcastle are still high. It is desirable for further studies to incorporate soil lead levels and investigate the association with DLLs.
2. With regard to other lead sources, specific measures such as actual amount of tap water drunk daily, lead levels in water and number of cigarettes smoked were not directly tested or analysed for lead concentration. Future research should record amount of water drunk per day, and lead concentrations in tap water should be sampled and analysed. This could determine whether tap water is a potential source of lead exposure, and if this is a source

of exposure could help local authorities to develop appropriate methods in order to reduce lead exposure.

3. The sample size may have been too small to detect associations with some determinants of DLLs, despite this study being large compared to other studies of teeth.

4. There is a possibility of selection bias due to low response rates from parents in more deprived areas. The lowest mean DLLs were found in children who lived in the most deprived areas, which was contrary to expectation.

5. The data obtained through interviews and/or questionnaires on the history of the participants may have been subject to recall bias or reporting bias, for example, when parents answered the question, “Has your child’s home ever had lead water pipes?” A review of the literature suggests that the extent of inaccurate recall may be related to the characteristics of the exposure and of respondents, although a distinction must be drawn between recall which is biased and that which is simply inaccurate. Interviewing techniques and the study protocol, including the design of questionnaires, as well as the motivation of respondents, play a central role and can to some extent be controlled by the investigator. The results of validation studies carried out to date suggest that the likelihood of recall bias may be greater when recall is poor in general. Recall bias is likely to affect the power of the study and the effect size observed although the direction of this effect is difficult to predict in situations of differential recall bias.

6. The Tooth Fairy study relied on data reported by parents who may have had little knowledge about human exposure to lead, and may not have been able to identify lead sources and hazards in their houses and environment. However, on the other hand this would have also meant that they were less likely to over report exposure.

7. Determinants were considered simultaneously, whereas possible concurrent interaction should be taken into account. Larger samples would be required to consider possible interaction between determinants of lead exposure. However, the Tooth Fairy Study focused only on the separate interaction of lead with each known determinant.

## **5.1.5 Strengths and limitations of the histological study**

### **Strengths**

#### **Histological strategy**

This is one of only a small number of studies of lead levels in different parts of the tooth, and it is unique in assessing lead in deciduous dentine. The present study was designed to investigate the underlying distribution of lead within dentine after accounting for variations in mineralisation and its timing to reveal the pattern of lead exposure over the life time of the tooth. From this work, I have shown that lead concentrations in primary dentine can be used as biomarkers of exposure in early life, and that this technique should be useful in investigating environmental factors relevant to lead contamination in order to protect health. It represents a better method than using single BLLs which merely reflect recent exposure.

#### **Analytical methods**

1. One of the important strengths of the present study is the measurement of lead levels in teeth using LA-ICP-MS. This is an accurate, high-quality technique for trace element measurement and allows a time-line of exposure to be determined. Unlike other techniques, this method does not completely destroy the tooth structure, and so samples can be analysed repeatedly.
2. Another important contribution of this study is the assessment of intra- and inter-individual variability in levels of lead showing how consistent lead ratios in teeth from the same child and at the same age interval in different ablation transects within the same tooth.
3. The present study indicated that consistent ratios at the same age interval were observed no matter where the sample was taken from the primary dentine, so that one ablation transect can be applied to examine the history of lead exposure. In future this will reduced analysis time and thereby reduce unit costs.
4. Due to using extracted or naturally shed teeth, we could not use carious teeth in the histological studies. However, our biomarker will be applicable to the majority of children/teeth in any given population.

5. GIS was used to map lead intensities and reveal patterns of lead exposure. When the timing of incorporation of lead into the enamel and dentine is identified, interpolated surface maps can be used. These can reveal differences in the distribution of calcium-normalised lead intensities related to age. GIS maps can also demonstrate lead intensities on different areas of the surface of the tooth along the ablation points.

## **Limitations**

### **Statistical power**

1. I planned to collect paired teeth from 15 children in order to analyse intra- and inter variability of lead between children. However, due to tooth caries, only 15/30 eligible teeth were able to be used in the histological part of the study. This is clearly small sample, however the volume of data from each tooth means that indicative results were produced. Although a sample was small, it was in fact larger than that of an important study undertaken in Australia by Arora et al (2006). [13]

2. Different lead ratios at the same age in primary dentine were found in some sections, probably due to the oblique section. In this case I found that transects in both teeth were not sampled along the dentine tubules resulting measurement error.

### **Sampling strategy and interpretation**

The lead ratios in secondary dentine are high and their interpretation remain unclear. Due to the interruption in dentine formation during the period of root formation, secondary dentine cannot be considered a simple continuation of primary dentine deposition. In controlling the transfer of mineral ions to the predentine layer, odontoblast activity is temporarily suspended. The incorporation of trace elements into the crystal apatite is different between these areas. As this stage it is assumed that the magnitude of lead levels in secondary dentine are due to the slower growth of the hydroxyapatite.

### **Quality of the tooth section**

1. The teeth from the Teesside sample were extracted due to being carious. In carious teeth, the demineralisation process may influence the mineral content of the dentine, which may potentially influence the lead levels found, especially in dentine close to the pulp cavity. In addition, carious teeth, especially in child HT6 who had the highest BLL,

meant the association between tooth and blood lead levels was difficult to assess. I was not able to measure the age of each ablation pit for this child, meaning I lost the opportunity to explore the history of lead exposure in the child having highest BLL.

2. Teeth were lapped down to 100µm sections in order to observe the histological detail via microscope. Some sections broke and were fixed using glue. If this fix did not match perfectly, the direction of dentinal tubules and the identification of incremental lines were difficult, potentially resulting in measurement error.

### **Lack of past history of the children**

No data was available about the history of lead exposure in the Teesside sample. Therefore, it was impossible to assess the associations between tooth lead concentration and any known risk factors. Such studies should be carried out in future.

### **Cost**

Although the LA-ICP-MS technique provides useful information about lead levels in different parts of the tooth, it is an expensive process compared to other techniques used in the past such as AAS. In addition, the histological technique is very time consuming and expensive which limits its applicability in large scale epidemiological studies.

## **5.2 Conclusions**

### **5.2.1 Epidemiological study**

Determinants of lead exposure were investigated in order to assess possible factors influencing dentine lead levels of children living in an historically-industrial area. Dentine lead levels were low (0.06-0.77ug/g) and generally in agreement with those found in other studies. Demographic variables such as age and sex were not associated with DLLs. Socio-economic variables, pica, age of housing, lead pipes and tap water consumption had no relationship with DLLs in the present study. It was not possible to study the influence of soil lead levels on DLLs but it is known that there are high lead levels in the soil in areas near the residences of some of the children. In the light of the current evidence on the toxicity of lead in children further work to characterise lead exposure in the UK is still required. Dentine lead offers an opportunity to study histories of exposure

in greater detail and should be developed further. Based on these findings, a histological technique was developed for assessing the history of lead exposure using DLLs.

### **5.2.2 Histological study**

From the histological study I concluded that:

1. Primary dentine is a suitable matrix to reconstruct the history of early childhood lead exposure. Lead ratios in primary dentine were consistent both between teeth from the same child and at the same age within each tooth, indicating that the history of exposure may be determined using a single ablation transect in primary dentine in a good quality section. The findings from child HT3 indicate that our technique can be used to identify history of lead exposure.
2. The cross striations in enamel ranged between 3.04-3.97 $\mu\text{m}$  (mean $\pm$ SD=3.5 $\pm$ 0.3). In dentine, the von Ebner lines, known as short-period incremental lines were between 2.75  $\mu\text{m}$  and 3.01  $\mu\text{m}$  (mean $\pm$ SD=2.9 $\pm$ 0.08).
3. Primary dentine could be used to measure lead exposure over a period of 1,000 days (almost 3 years), a far longer period of exposure than ELLs (~240days), or BLLs (~30days).
4. Interpretation must distinguish between primary and secondary dentine. Lead ratios in primary dentine were 2-5 times lower than secondary dentine. The normalized lead ratios across the tooth surfaces observed in this study follow the order of secondary dentine > primary dentine > postnatal enamel > prenatal enamel.
5. Significantly higher levels of lead were found at the dentine near the pulp cavity. Variations in mineralization in the different regions of dentine may lead to such a differential incorporation of lead ratios. However these high lead concentrations were found in a small number of teeth (6 teeth) taken from 4 children. Therefore, a relationship between BLLs and lead concentrations in secondary dentine could not be conclusively demonstrated due to the small of sample size.
6. The spatial distribution of lead can be visualised using GIS.

7. BLLs were low, in agreement with several studies which showed a major decrease of BLLs following the reduction in lead in many sources. However, child HT3 shows a higher tooth lead ratio and higher BLL.

8. The findings of the present study suggest that LA-ICP-MS is a useful technique for the determination of trace element distribution in the dental tissues which calcify at different times during development.

The following sections summarise the answers to each of my research questions.

**Question 1:** Are mean dentine lead levels associated with known determinants of lead exposure?

**Answer:** Unlike other studies, the results in the Tooth Fairy Study did not find a clear relationship between determinants of lead exposure and DLLs in children living in Newcastle. The findings may point towards a lower bioavailability of lead via possible routes such as dust, food and soil exposure pathways in this city.

**Question 2:** Can our histological technique recreate the history of lead exposure over a child's life time?

**Answer:** The technique we developed is suitable to be used in identifying the history of lead exposure through early life.

**Question 2a:** Should we sample enamel and/or dentine?

**Answer:** Dentine provides the opportunity to establish a detailed chronology of incorporation of lead over a longer period than enamel.

**Question 2b:** How many transects per tooth are required?

**Answer:** Based on results in a small set of good tooth sections, in primary dentine, one transect may provide the pattern of lead ratios which are consistent at the same age within each tooth, indicating that the history of lead exposure may be determined using a single, multi-point ablation transect on longitudinal sections of individual teeth. However, we recommend further validation this finding in larger population samples, including populations with known lead exposure.

**Question 2c:** How many teeth per child are required?

**Answer:** The results showed that differences in lead ratios between good sections in two teeth from the same child were small although we do not provide sufficient evidence that one tooth is sufficient. We recommend an expansion of this study with a larger number of samples and the collection of multiple teeth from each of the participants to allow refinement and confirmation of the histological technique proposed in the present study.

**Question 2d:** Does tooth type matter?

**Answer:** Tooth type appears not to be a critical in determining variations in lead ratios in the present study, although we only analysed one tooth type (molar) so recommend further study in multiple tooth types to explore potential variations of lead levels between different tooth types.

**Question 3:** What is the relationship between tooth and blood lead levels?

**Answer:** There was no statistically significant association between tooth and blood lead levels in the Teesside samples, and further work will be required in a bigger sample to establish any link.

## 5.3 Recommendations

Environmental lead exposure remains one of the most common paediatric environmental health problems in many countries including the USA. Attempts have been made to reduce environmental lead levels, and while successful in driving down the number of children having high lead levels, lead exposure remains a public health issue.

The following sections provide recommendations in order to decrease exposure to lead in children.

1. More studies are needed on exposure to lead in younger children.
2. The scientific committee on neurotoxicology and psychophysiology and the scientific committees on the toxicology of metals of the international commission on occupational health (ICOH) have suggested that BLLs should be reduced worldwide to 5µg/dL, and that a further revision should be carried out in the future since lead toxicity remains at even lower levels than this value. This is in agreement with the latest CDC update (July 2012) suggesting a revised reference BLL value of 5µg/dL. [371] I agree with Gilbert et



al (2006) who suggested that the BLL action value in children should be lowered to 2µg/dL. [372]

3. Blood and tooth samples should be taken to assess lead exposure of children living in contaminated areas.

## **5.4 Direction of future work**

It is evident from the present study that this new technique will greatly aid future studies exploring lead exposure histories, identifying key exposure sources and vulnerable life stages. The following directions of future work are suggested:

1. An implication of these findings is that this technique is suitable to be used in designing epidemiological studies such as the history and determinants of metal exposure. Trace element exposure occurs differently depending on differences in exposure patterns among populations, sampling technique used, and durations of exposure. Most of the teeth in the histological study showed low lead levels with the same pattern. Lead ratios increased gradually with age. As stated before, however, interesting findings emerged for child HT3. The levels of lead in this child changed, implying changes in the degree of lead exposure; it is worth pointing out that this child with a higher BLL also had higher tooth lead levels. Building on the epidemiological study, it will be important to undertake epidemiological studies to identify causes and factors influencing lead exposure over the early life course in areas of different exposure. A better understanding of the time gap between the cessation of primary dentine formation and secretion of secondary dentine is important. Historical record of childhood exposure are needed to link them to tooth lead levels.
2. Investigating dentine lead levels in samples taken from populations in highly-polluted areas will be useful in confirming this approach to identify the history of lead exposure.
3. Collection of multiple teeth from participants will allow the study of variations of lead levels among different types of teeth to be further explored.
4. It will be interesting to apply this method to other trace elements, for example in manganese. Manganese is involved in several industrial activities such as mining and steel work; therefore the methods developed in the present study could be applied to

explore the history and determinants of manganese exposure in order to help public health surveillance.

5. Levels of several other elements (e.g. Pb, Mg, P, Cl, Ca, Fe, Zn, Sr and Ba) can be measured simultaneously via LA-ICP-MS. The study of other elements examined could provide further insight into variations in the diets as well as potential sources of environmental pollution. It will be crucial to investigate variations in tooth lead levels alongside iron levels because several studies have indicated that elevated lead levels may be found in cases of iron deficiency.

6. I hope to apply the technique developed here in order to study the history of lead exposure in Thai children from highly-polluted lead mining areas. Although the plants are now closed, people living around the contaminated areas are still exposed to lead from several pathways such as water, soil and crops. Given the robust methodology, I hope that I will be able identify critical sources of exposure and vulnerable life stages to enable me to produce useful public health advice to reduce exposure in future generations.

Over the past two decades, lead concentrations in the atmosphere have decreased dramatically around the world. This is because many countries have banned and removed tetraethyl lead from petrol. [373] Nevertheless, humans can be exposed to lead from several other sources such as food, water, house dust, soil and industrial activities. Environmental exposure to lead is therefore an important issue of continuing public health concern. The bio-monitoring of human lead exposure reflects individual body burden and can reveal key sources of past or current lead exposure. [5] Thus, the appropriate choice and reliable measurement of biological markers is crucial for preventing exposure and public health decision making.

The main purpose of this thesis was to develop a method to assess the history of lead exposure using tooth lead levels to reveal exposure over a child's lifetime. I developed a novel technique to date ablation points in dentine in milk teeth, and, using this technique have demonstrated that primary dentine is a potential biomarker for characterising the early life history of lead exposure in children.

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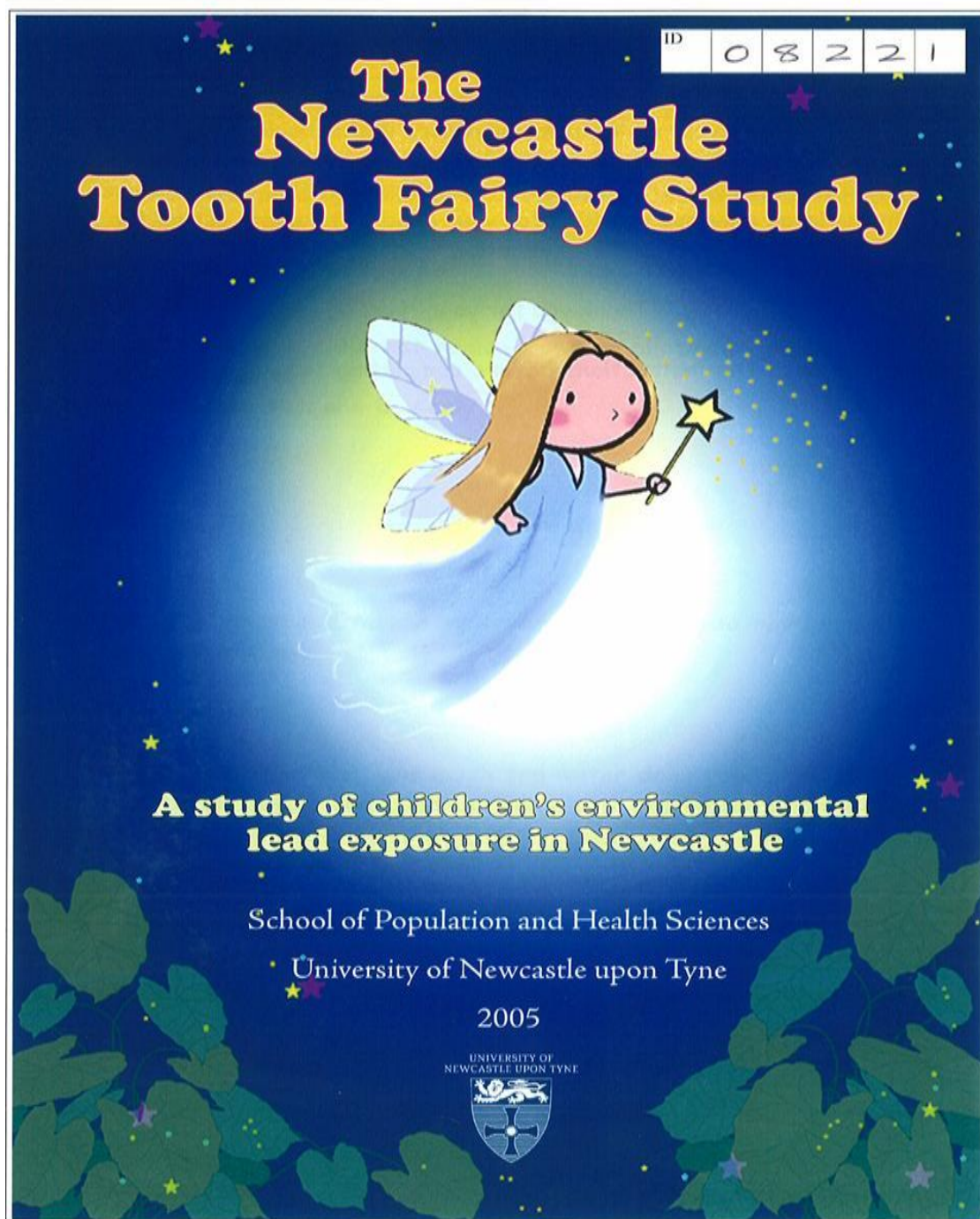
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# APPENDICES

## APPENDIX I:

### The Newcastle Tooth Fairy Study's questionnaire



Your reply will be kept in strict confidence. There is no need to write your child's name anywhere in this booklet

When you have answered the questions, please put this questionnaire in the large envelope provided for your child to take into school for collection

Thank you for your time

Please read the instructions before completing the questionnaire



## How to answer the questions

There are several types of question in this booklet. Most of them can be answered by ticking a box. Please use blue or black ink to fill in your answers.

*For example:*

Q. Does your child eat home-grown or allotment-grown vegetables?

Please tick one box.

Yes ..... ☒

No ..... ☐

*Some of these questions have several boxes and you may be asked to tick one box only.*

*For example:*

Q. Approximately, how much tap water does your child drink each day including drinks made with tap water and filtered water?

Please tick one box only.

None ..... ☐

Up to ½ pint ..... ☐

½ pint to 1 pint ..... ☐

More than one pint ..... ☒

*Another type of question asks you to write your answer on a line or in a space provided*

*For example:*

Q. What is your child's date of birth?

Please answer on the line below.

Day                      Month                      Year

---

*Some questions ask you to tick more than one box*

*For example:*

Q. Which of the following educational establishments has the main wage earner in the household attended?

Please tick all that apply.

Primary or secondary school .....☒

College of Further Education or other type .....☒  
of college

Polytechnic or University (including Open .....☐  
University)

Or to write a number in a box

*For example:*

Q. *How long has your child lived in this place of residence?*

*Please complete the box below*

Years

If you need any help filling in the questionnaire, please contact-

Miss Laura Stokoe

Research Secretary

School of Population and Health Sciences

The Medical School

University of Newcastle

Newcastle upon Tyne

NE2 4HH

Telephone 0800 7318848

Thank you, now please start at Q 1.1 on the next page

<b>1. About your child</b>
----------------------------

Q 1.1 Is your child male or female? Please tick one box only.

Male ..... ☐ 1  
Female ..... ☐ 2

Q 1.2 What is your child's date of birth? Please answer on the line below.

Day Month Year

.....

Q 1.3 Please refer to the map on the following page which shows Newcastle city boundaries. Has your child lived in Newcastle upon Tyne since birth? Please tick one box.

Yes ..... ☐ 1  
No ..... ☐ 2

*If you have answered 'No' please answer Q 1.4.  
If you have answered 'Yes' please go straight to Q 1.5*

Q 1.4 How many years has your child lived outside of Newcastle City boundaries? Please give the number of years.

Years

Q 1.5 Please give the name and address of your child's GP/Practice

Please write on the lines below.

GP name/Practice name

.....

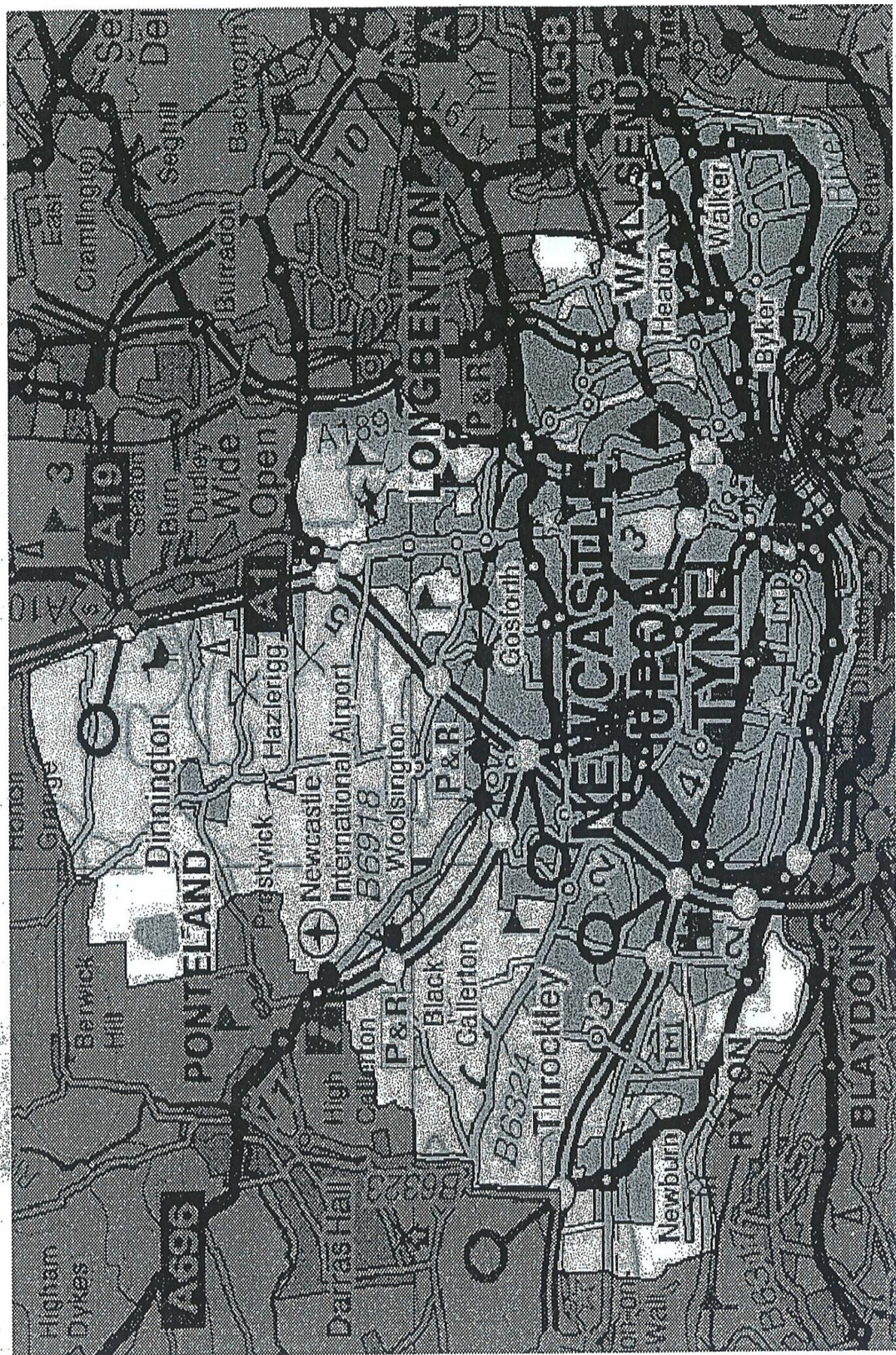
Address

.....

Postcode

.....







## 2. Your child's current main place of residence

This section is about your child's current main place of residence.

Q 2.1 Please give the full postcode of your child's home

Please complete the boxes below.

--	--	--	--	--	--	--	--

Q 2.2 How many years has your child lived in this place of residence?

Please complete the boxes below.

--

 Years

Q 2.3 Does your child's home have lead water pipes? Please tick one box only.

Yes	.....	<input type="checkbox"/>	1
No	.....	<input type="checkbox"/>	2
Not sure	.....	<input type="checkbox"/>	3

If you have answered 'No' or 'Not Sure' please answer Q 2.4.

If you have answered 'Yes' please continue to Q 2.5.

Q 2.4. In your child's lifetime, has your child's home ever had lead water pipes?

Please tick one box only.

Yes	.....	<input type="checkbox"/>	1
No	.....	<input type="checkbox"/>	2
Not sure	.....	<input type="checkbox"/>	3

If you have answered 'Yes', please give the year that the lead pipes were replaced. If you are not sure about the year, please give the year you think they were replaced. Please complete the boxes below.

--	--	--	--

Q 2.5 How often is your house vacuumed? Please tick one box only.

More than once per week	.....	<input type="checkbox"/> 1
Weekly	.....	<input type="checkbox"/> 2
Less than weekly	.....	<input type="checkbox"/> 3

Q 2.6 Do you have hard floors in the house (apart from the bathroom and kitchen)?

Please tick one box only.

Yes	.....	<input type="checkbox"/> 1
No	.....	<input type="checkbox"/> 2

If you have answered 'Yes' please answer Q 2.7.

If you have answered 'No' please continue to Q 2.9.

Q 2.7 How often are the hard floors in your child's home cleaned (either dry cleaned with a dust pan and brush or broom, or vacuum/wet cleaned with a mop)? Please tick one box only

More than once per week	.....	<input type="checkbox"/> 1
Weekly	.....	<input type="checkbox"/> 2
Less than weekly	.....	<input type="checkbox"/> 3

Q 2.8 Are there hard floors usually dry cleaned or vacuum/wet cleaned? Please tick one box only.

Dry cleaned with a dust pan and brush or broom	.....	<input type="checkbox"/> 1
Vacuumed/Wet Cleaned with mop	.....	<input type="checkbox"/> 2
Both	.....	<input type="checkbox"/> 3

Q 2.9 When was the house built, not including renovations or additions? If you are not sure, please give the year you think it was built.

Please complete the boxes below.

--	--	--	--

Q 2.10 What is the general condition of the interior paintwork including paint on walls, window sills, skirting boards, doors etc?

Please tick one box only.

- Paint is in good condition with no flaking or peeling ..... ☐1
- There is some flaking or peeling ..... ☐2
- No Paint ..... ☐3

Q 2.11 Was the house built before 1960?

Please tick one box only.

- Yes ..... ☐1
- No ..... ☐2

If you have answered 'Yes' please answer Q 2.12.

If you have answered 'No' please read the instruction after Q 2.12.

Q 2.12 Have there been any renovations in your child's lifetime during which the following processes have taken place? Please tick one box only.

- Old paint (interior or exterior) has been burnt off, sanded or water blasted ..... ☐1
- Old paint (interior or exterior) has been stripped with chemicals ..... ☐2
- Ceiling, floors and walls have been demolished ..... ☐3
- None of these processes ..... ☐4

We may have sent you up to two additional sections to fill in, depending on how you answered the questions we asked when you initially called the Tooth Fairy Study phone line.

If either Section 2B (on pink paper) or Section 2C (on blue paper) were sent to you, please fill them in. If not, please continue to Section 3.

Section 2B (on pink paper) is about any other homes or places that your child frequently visits e.g. houses of another parent, grandparents, friends, other relatives or childminders. If your child visits one of these places for more than ten hours a week please fill in this section.

Section 2C (on blue paper) is about previous main places of residence e.g. your previous home, or previous places your child used to frequently visit e.g. previous homes of another parent, relatives or previous childminders. If your child has previously lived in another house or used to frequently visit another house please fill in this section.

### 3. About what your child eats and drinks

Q 3.1 Do you, or somebody your child visits frequently, grow produce in an allotment plot or in the house garden? Please tick one box only.

Yes ..... ☐

No ..... ☐

If you have answered 'Yes' to this question please answer question 3.2

If you have answered 'No' please go straight to question 3.3

Q 3.2 Does your child eat the vegetables that are grown?

Please tick one box only.

Yes ..... ☐

No ..... ☐

Q 3.3 Has your child ever been diagnosed with a calcium or iron deficiency? Please tick one box only.

Yes ..... ☐

No ..... ☐

Not sure ..... ☐

Q 3.4 Approximately, how much tap water does your child drink each day including drinks made with tap water and filtered water?

Please tick one box.

None ..... ☐

Up to ½ pint ..... ☐

½ pint to 1 pint ..... ☐

More than one pint ..... ☐



Q 3.5 As a baby did your child ever receive bottle feeds (apart from expressed milk)?  
Please tick one box only.

Yes ..... ☐

No ..... ☐

If you have answered 'Yes' to this question please answer Q3.6

If you have answered 'No' please go straight to question Q4.1

Q 3.6 If yes, from what age? Please fill in the age in the boxes below.

*Weeks*

--

*Months*

--

#### 4. About other aspects of your child's eating behaviour

Q 4.1 Does your child suck his/her thumb? Please tick one box only.

Yes Currently	.....	<input type="checkbox"/>
Not currently but has done so in the past	.....	<input type="checkbox"/>
No and never has	.....	<input type="checkbox"/>

Q 4.2 Does your child chew or mouth painted objects? Please tick one box only.

Yes Currently	.....	<input type="checkbox"/>
Not currently but has done so in the past	.....	<input type="checkbox"/>
No and never has	.....	<input type="checkbox"/>
Not sure	.....	<input type="checkbox"/>

Q 4.3 Does your child suck on hair or clothing? Please tick one box only.

Yes Currently	.....	<input type="checkbox"/>
Not currently but has done so in the past	.....	<input type="checkbox"/>
No and never has	.....	<input type="checkbox"/>
Not sure	.....	<input type="checkbox"/>

Q 4.4 Does your child put small toys or other objects such as buttons in his/her mouth?  
Please tick one box only.

Yes Currently	.....	<input type="checkbox"/>
Not currently but has done so in the past	.....	<input type="checkbox"/>
No and never has	.....	<input type="checkbox"/>
Not sure	.....	<input type="checkbox"/>

Q4.5 Does your child eat soil, dirt or any creatures that live in the soil such as earthworms? Please tick one box only.

Yes Currently	.....	<input type="checkbox"/>
Not currently but has done so in the past	.....	<input type="checkbox"/>
No and never has	.....	<input type="checkbox"/>
Not sure	.....	<input type="checkbox"/>

Q 4.6 Does your child suck, chew or eat paper? Please tick one box only.

Yes Currently	.....	<input type="checkbox"/>
Not currently but has done so in the past	.....	<input type="checkbox"/>
No and never has	.....	<input type="checkbox"/>
Not sure	.....	<input type="checkbox"/>

Q 4.7 Does your child suck chew or eat pencils or any other writing implements? Please tick one box only.

Yes Currently	.....	<input type="checkbox"/>
Not currently but has done so in the past	.....	<input type="checkbox"/>
No and never has	.....	<input type="checkbox"/>
Not sure	.....	<input type="checkbox"/>

## 5. About the family at your child's main place of residence

**This section is about the family at your child's main place of residence.**

Q 5.1 Including yourself, how many people live in your household?

Please write on the lines.

Number of adults (aged over 16 years) \_\_\_\_\_

Number of children aged 5 and over \_\_\_\_\_

Number of children under 5 \_\_\_\_\_

Q 5.2 Many jobs or hobbies can involve the use of lead. Do any household members in the main place of residence take part in any of the following activities?

Please tick all that apply.

Car repair	..... <input type="checkbox"/>
Panel-beating	..... <input type="checkbox"/>
Lead lighting	..... <input type="checkbox"/>
Lead window making	..... <input type="checkbox"/>
Lead weight making	..... <input type="checkbox"/>
Electronics	..... <input type="checkbox"/>
Ammunition handling	..... <input type="checkbox"/>
Car battery service/repair or manufacture	..... <input type="checkbox"/>
Floor sanding	..... <input type="checkbox"/>
Other	..... <input type="checkbox"/>
Please specify	_____

Q 5.3 Does your child have a brother or sister, or playmate with a known elevated blood lead level?

Please tick one box only.

Yes	..... <input type="checkbox"/>
No	..... <input type="checkbox"/>

Q 5.4 Does anyone smoke in the main place of residence?

Please tick one box only.

Yes	..... <input type="checkbox"/>
No	..... <input type="checkbox"/>

Q 5.5 Have your child or any member of the household ever use kohl (also known as kajal, al-kahl, or surma) eyeliner?

Please tick one box only.

Yes ..... ☐  
No ..... ☐

Q 5.6 Would you please tell us which group below best describes your total HOUSEHOLD income after tax and deductions (i.e. take home pay)? Please include any allowances, benefits or pensions you or any members of your household receive.

Please tick one box.

Total take home pay per week	Total take home pay per month	
Up to £47 per week	Up to £208 per month	..... <input type="checkbox"/>
£48-£96 per week	£209-£416 per month	..... <input type="checkbox"/>
£97-£192 per week	£417-£833 per month	..... <input type="checkbox"/>
£193-£288 per week	£834-£1249 per month	..... <input type="checkbox"/>
£289-£384 per week	£1250-£1666 per month	..... <input type="checkbox"/>
£385-£481 per week	£1667-£2083 per month	..... <input type="checkbox"/>
£482-£577 per week	£2084-£2499 per month	..... <input type="checkbox"/>
Over £578 per week	Over £2500 per month	..... <input type="checkbox"/>

Q 5.7 Which of the following educational establishments has the main wage earner in the household attended?

Please tick all that apply.

Primary or secondary school	..... <input type="checkbox"/>
College of Further Education or other type of college	..... <input type="checkbox"/>
Polytechnic or University (including OU)	..... <input type="checkbox"/>

We may have sent you one additional section concerning the people your child lives with. This depends on how you answered the questions we asked when you initially called the Tooth Fairy Study phoneline.

If this additional section was sent to you, please fill them in.

Section 5b (on green paper) is about the people your child may stay with frequently. Please answer these questions if your child visits any other home e.g. the home of another parent, grandparents, child minders etc, more than 10 hours per week

# APPENDIX II: CONSENT FORM

**Version 2.0 Date 8 May 2009**

**Lead levels in teeth as a measure of cumulative lead exposure in children**

**Protocol Number: 09/H0905/42**

**Principal Investigator:** Dr Susan Hodgson

**Co-investigator:** Miss Charuwan Manmee

**Participant details:**

**Gender:**

**Age:**

By signing this document you consent to your child's participation in the research project '**Lead levels in teeth as a measure of cumulative lead exposure in children**' being undertaken by Miss Charuwan Manmee at Newcastle University. Please tick to confirm that:

You have read the consent form and explained to your child what the study will involve ☐

Your questions relating to this research project have been answered ☐

You have understood the patient information sheet dated 8 May 2009 (version 2.0.) and have decided, with your child, to participate in this study ☐

You understand that your child's participation is voluntary, and that you and your child are free to withdraw at any time without your medical care or legal rights being affected ☐

You understand that if we find your child's teeth to be very unsound upon removal we may not be able to analyse these teeth, and your child may be excluded from our analysis ☐

\_\_\_\_\_  
Signature of Parent/Legal Guardian (if necessary)

\_\_\_\_\_  
Date

\_\_\_\_\_  
Signature of Investigator

\_\_\_\_\_  
Date

\_\_\_\_\_  
Signature of Witness (if appropriate)

\_\_\_\_\_  
Date

All of the information you provide to Miss Manmee will be kept confidential.

A copy of the informed consent will be given to you for your records.

# APPENDIX III: PUBLICATIONS

1. Shepherd TJ, Dirks W, Manmee C, Hodgson S, Banks DA, Averley P, Pless-Mulloli T. **Reconstructing the life-time lead exposure in children using dentine in deciduous teeth.** *Science of the Total Environment* 2012;425:214-222

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## Reconstructing the life-time lead exposure in children using dentine in deciduous teeth

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### ABSTRACT

Data are presented to demonstrate that the circumpulpal dentine of deciduous teeth can be used to reconstruct a detailed record of childhood exposure to lead. By combining high spatial resolution laser ablation ICP-MS with dental histology, information was acquired on the concentration of lead in dentine from *in utero* to several years after birth, using a true time template of dentine growth. Time corrected lead analyses for pairs of deciduous molars confirmed that between-tooth variation for the same child was negligible and that meaningful exposure histories can be obtained from a single, multi-point ablation transect on longitudinal sections of individual teeth. For a laser beam of 100 µm diameter, the lead signal for each ablation point represented a time span of 42 days. Simultaneous analyses for Sr, Zn and Mg suggest that the incorporation of Pb into dentine (carbonated apatite) is most likely controlled by nanocrystal growth mechanisms. The study also highlights the importance of discriminating between primary and secondary dentine and the dangers of translating lead analyses into blood lead estimates without determining the age or duration of dentine sampled. Further work is in progress to validate deciduous teeth as blood lead biomarkers.

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### 1. Introduction

The intrinsic importance of teeth as historical records of lead exposure has been recognised, tested and discussed for several decades. From the seminal papers of Rabinowitz et al. (1991, 1993) to the more recent works of Arora et al. (2011, 2006) and Hare et al. (2011) through the wealth of isotope studies by Gulson and Wilson (1994), Gulson (1996), Farmer et al. (2006) and Robbins et al. (2010), research has revealed the value of dental enamel and dentine as biological indicators of exposure. Central to many of these studies has been the aim to develop a blood lead biomarker as an alternative to blood lead measurements (BPb). The latter are still the most reliable indicator of recent lead exposure, both for screening and for bio-monitoring purposes. For periods longer than about 40 days however, the half life of lead in the blood compartment, serial sampling is required. As succinctly stated by Barbosa et al. (2005) 'It appears impossible to differentiate between low-level chronic Pb exposure and a high-level short Pb exposure based on a single BPb measurement'. By contrast, deciduous teeth are easy to collect (i.e. require non-invasive

procedures), chemically stable and in theory provide a continuous record from *in utero* to several years after birth. Unfortunately, previous work has not had the desired degree of control over the temporal relationship between blood sampling and that part of the tooth corresponding to the exact time of sampling. This failure has been exacerbated by the different analytical tools employed, as a result of which, published tooth data have proven hard to interpret (Grobler et al., 2000). Recent studies (Arora et al., 2006) have sought to resolve this problem using the neonatal line as a fixed point in time. Whilst advancing our understanding, there remain issues to address concerning the processes governing the incorporation of lead into dental tissues and the stability of lead once incorporated.

This paper describes our research on the dentine in a small set of deciduous teeth to reconstruct the childhood history of lead exposure using laser ablation ICP mass spectrometric analysis for lead (LA-ICP-MS) combined with precise age measurements derived from the histological examination of dentine. Until recently, dentine was referred to in the literature as 'hydroxylapatite' and modelled using the stoichiometric composition  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ . Biological apatites (in bone and dentine) differ however in several important respects, including non-stoichiometry, small crystal dimensions and a high degree of structural disorder (Boanini et al., 2010). Furthermore, the marked reduction in OH bonds and the substitution of 5–6 wt.%

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**Table 1**

Deciduous teeth selected for analysis. Teeth shown in *italics* were unsuitable for histological examination.

Child ID	Tooth ID		Age of child (years)
	Tooth 1	Tooth 2	
HT1	55	54	6
HT2	54	64	7
HT3	55	65	7
HT5	65	54	8
HT6	(65)	(74)	8
HT8	(84)	85	7
HT9	55*	65*	6
HT11	85	64	6
HT12	85*	84	8
HT13	75*	84	8
HT14	74	84*	7

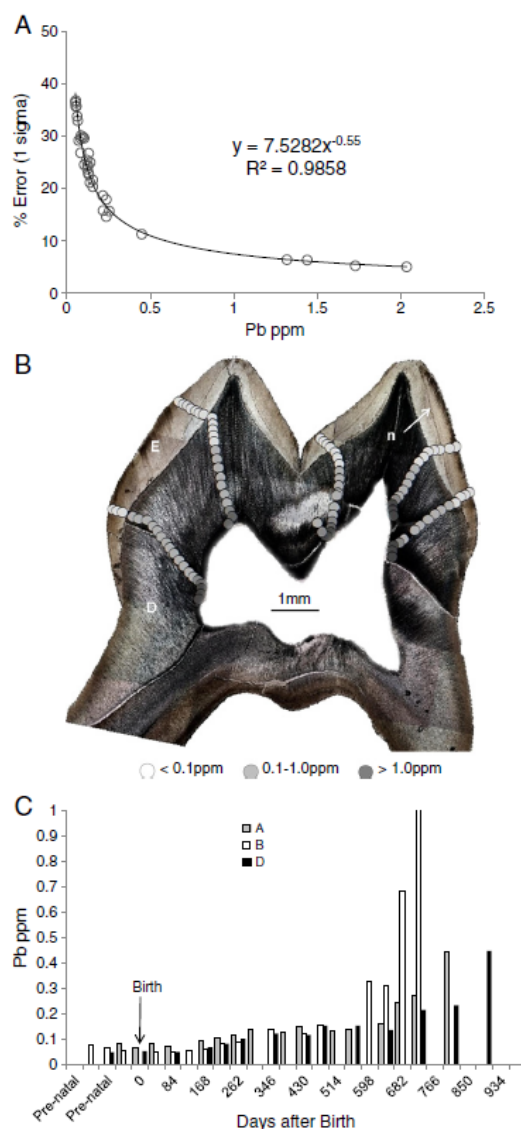
(NB. Samples marked with an asterisk did not expose the pulp cavity in longitudinal section.)

(CO<sub>3</sub>)<sup>2-</sup> for (PO<sub>4</sub>)<sup>3-</sup> in bioapatites, suggests that dentine should be more correctly described as nanocrystalline 'carbonated apatite' (Pasteris et al., 2004). We welcome this suggestion and encourage its general adoption. Informed by new research on the synthesis of carbonated bioapatites we have also acquired matching data for Zn, Sr and Mg (see Section 5. Discussion). These three elements have very different biological roles from Pb but together help to explain and validate the spatial and temporal patterns we have observed for lead in dentine.

LA-ICP-MS is now an accepted technique for the analysis of dental tissues and with continued improvements in instrument design and the availability of affordable lasers, the elemental analysis of teeth can be performed routinely (Arora et al., 2011; Hare et al., 2011). Though the technique may be regarded as mature, the reproducibility and accuracy of analysis depend upon the type of laser employed and the choice of appropriate standard reference materials. Our work builds upon important advances in laser ablation methodology as reported by Delmdahl and von Oldershausen (2005) and Guillon et al. (2003).

Dentine is described as *coronal* or *root* depending on which part of the tooth is indicated. Within coronal dentine (i.e. the crown of the tooth), *primary dentine* is the first formed and constitutes the bulk of the coronal dentine. It comprises a layer of *mantle dentine*, about 20 µm in thickness following the enamel-dentine junction, upon which primary dentine is secreted incrementally; the latter being referred to as *circumpulpal dentine*. After root formation is complete, a thin layer of *secondary dentine* may form in continuity with the primary dentine but distributed more unevenly around the pulp cavity. As discussed later, it is important to realise there is a significant time lag between the completion of primary and onset of secondary dentine. In response to tooth attrition or dental caries, a third type of dentine may form. Known as *tertiary dentine* it forms locally within the circumpulpal dentine and is intended to block the dentinal tubules; reactive in the case of attrition, or

reparative in the case of caries (Linde, 1992). In this paper we refer only to primary and secondary dentine and for convenience have used the following abbreviations: EDJ-enamel/dentine junction; DPC-dentine/pulp cavity junction.



**Fig. 1.** a. Graph summarising the estimated % within-run errors (1 sigma) on lead measurements in dentine from 0.06 ppm to 2.0 ppm Pb. The regression equation was based on data for HT11–85 but is also valid for higher lead concentrations. b. Illustration to show the distribution of multiple laser ablation transects in the crown of tooth HT1–55. Each transect begins at the enamel surface, crosses the enamel-dentine junction and follows the line of the dentine tubules towards the pulp cavity. On reaching the pulp cavity each transect was extended laterally along the edge of the dentine to replicate the youngest layers of dentine. The symbols used for the ablation point have been enlarged to emphasise the line of the transects. Greyscale shading for individual ablation points generalises the overall lead concentration (ppm). Key: E-dentine; n-neonatal line in enamel (neonatal line in dentine not shown). c. Transects A, B and D for tooth HT11–85 showing the close agreement between individual time-adjusted lead concentration profiles in dentine.

**Table 2**

Laser and ICP-MS specifications and operating conditions.

Laser model	Geolas ArF eximer
Laser wavelength	193 nm
Laser energy	10 J/cm <sup>2</sup>
Laser pulse rate	5 Hz
Pulses per ablation analysis	200
Beam diameter	100 µm
ICP-MS model	Agilent 7500c quadrupole MS
Scanning mode	Peak jumping
Background acquisition time	~20 s
Signal acquisition time	~40 s
Dwell times	20 ms <sup>208</sup> Pb 10 ms <sup>24</sup> Mg, <sup>44</sup> Ca, <sup>66</sup> Zn, <sup>88</sup> Sr



## 2. Samples

The teeth were provided by a tertiary referral dental practice in Teesside, NE England (Queensway Dental Practice) and comprised 15 pairs of extracted deciduous molars (1st and 2nd molars). Ethical approval for the study was granted through the NHS County Durham & Tees Valley 1 Research Ethics Committee and full consent was obtained from both parents and children in all cases. Initially it was intended to compare all 15 pairs but the presence of dental caries and excessive attrition limited the preparation to 22 teeth (Table 1). After analysis, three pairs were subsequently judged unsuitable for histological examination and have been excluded from discussion. Tooth IDs refer to specific teeth in accordance with the two digit Fédération Dentaire Internationale primary dentition nomenclature (Hillson, 1996). Also shown in Table 1 is the age of the child at the time of tooth extraction. In accepting these teeth we had no reason to believe that the children selected for the study had been exposed to anything other than background levels of lead; consistent with an historic industrial region of the UK.

Samples were prepared for analysis as 150 µm thick, doubly polished, longitudinal slices of tooth as follows. Using a low speed saw, 500 µm thick longitudinal slices were cut from each tooth and mounted onto glass slides using dental wax. These were then ground down to approximately 150 µm and polished with 1 µm alumina powder. The slices were then removed, flipped over, remounted and the second surface polished. Small changes in procedure were adopted to prepare the samples for histological examination (e.g. thinning to 100 µm) but otherwise the methodologies were broadly similar.

## 3. Methods of analysis

### 3.1. LA-ICP-MS analysis

Trace element analysis of the teeth was performed using a GeoLas 193 nm ArF excimer laser coupled to an Agilent 7500c ICP-mass spectrometer at the School of Earth and Environment, University of Leeds, UK. At 193 nm, most materials photoabsorb leading to rupture of the chemical bonds without thermal expansion or damage to the surrounding material (Delmdahl and von Oldershausen, 2005). Guided by histological criteria, a series of 100 µm diameter ablation pits were made at intervals of 100–200 µm along transects extending from the enamel surface, across the EDJ to the DPC for each tooth.

During ablation, the process was monitored via a video camera integrated into the optical array.

Using a spot size of 100 µm, a constant energy density of 10 J/cm<sup>2</sup> and a pulse rate of 5 Hz (Table 2), the aspect ratio (depth/diameter) of each ablation pit never exceeded 1; thus minimising potential element fractionation (Kosler, 2008). Subsequent optical examination of the ablation pits confirmed very flat bottoms, straight vertical sides and no evidence of surface spalling, consistent with controlled excimer excitation.

Ion intensities at isotope masses <sup>24</sup>Mg, <sup>44</sup>Ca, <sup>66</sup>Zn, <sup>88</sup>Sr and <sup>208</sup>Pb were recorded in time resolved scanning mode and converted into element/calcium ratios using data for standard reference materials analysed before and after each analytical session. Reference material NIST SRM Glass 610 was used for instrument calibration and cross referenced to replicate analyses of NIST SRM Glasses 612 and 614 to establish instrument performance and within-run standard errors. SRM Bone Meal 1486, having a matrix similar in chemical and mineralogical composition to dentine, was used as an external unknown. No apparent isobaric interferences were observed for the aforementioned elements. Minimum detection limits were calculated as 3 times the background count rates on the carrier gas blank before ablation. Routine detection limits for Mg, Zn, Sr and Pb were typically 0.03–0.09 ppm, 0.15–0.19 ppm, 0.002–0.004 ppm and 0.01–0.02 ppm respectively. The measured values for Sr and Pb were within 2–8% of the certified values for NIST 612 (78.4 ppm Sr; 38.57 ppm Pb) and NIST 614 (45.8 ppm Sr; 2.32 ppm Pb), and within 12–17% of the certified values for NIST 1486 (264 ppm Sr; 1.33 ppm Pb). For Zn and Mg, measured values were within 10–15% of the certified values for NIST 1486 (147 ppm Zn; 4660 ppm Mg). Certified data for Zn and Mg in NIST 612 and 614 are not available but the measured values are in close agreement with the working values published by Pearce et al. (1997).

Absolute concentrations of Mg, Sr, Zn and Pb in dentine were calculated by normalising to 26.5 wt.% Ca (Arora et al., 2006). Data processing was performed off-line using SILLS; a software programme specifically written for the signal integration of laboratory laser systems by Murray Allan (University of Leeds) and later modified by Dimitri Meier and Marcel Guillong (Die Eidgenössische Technische Hochschule, Zurich).

SILLS error estimates for lead analyses of dentine based on data for tooth HT11–85 are shown in Fig. 1a. These obey a power law

**Table 3**  
Data summary showing the minimum and maximum values for lead, zinc, strontium and magnesium in circumpulpal primary dentine.

Tooth ID	Pb ppm		Zn ppm		Sr ppm		Mg ppm	
	Min	Max	Min	Max	Min	Max	Min	Max
HT1–54 mean	0.06	1.79(3.00)	54	303(373)	40	75	1851	6855
HT1–55 mean	0.06	1.67(2.21)	51	292(344)	40	79	7249	11185
HT2–54	0.07	1.63	67	379	57	89	7338	10937
HT2–64	0.08	3.35	55	377	52	83	7633	12024
HT3–55	0.63	10.83	102	328	105	157	6679	11030
HT3–65	0.59	15.20	108	413	106	147	6962	11440
HT5–54	0.07	0.64	57	182	40	50	6840	10238
HT5–65	0.13	0.48	54	140	38	49	7515	10168
HT8–85	0.16	5.81	59	372	36	55	7007	11056
HT9–55*	0.06	0.23	63	89	34	41	7114	9230
HT9–65*	0.04	0.87	56	187	35	47	3065	6021
HT11–64 mean	0.06	1.97(3.37)	65	333(481)	49	66	6249	10096
HT11–85 mean	0.05	1.69(3.36)	57	290(404)	42	54	1156	8372
HT12–84	0.06	1.50	50	425	44	193	1772	4372
HT12–85*	0.08	0.27	63	100	38	50	7589	8696
HT13–75*	0.10	1.10	71	143	118	156	8775	9470
HT13–84	0.13	1.24(5.79)	75	139(314)	119	156	7597	10876
HT14–74	0.14	0.95	79	276	47	60	7484	9554

Mean values are for multiple laser transects on the same sample.

\*Refers to sections that did not cut the pulp cavity.

Values in brackets are for secondary dentine.



distribution and for a minimum concentration of 0.06 ppm Pb the calculated 1 $\sigma$  error is ~40%, decreasing to ~5% for a maximum concentration of 2.0 ppm Pb.

A limitation we noted when using NIST 1486 as a primary reference material for LA-ICP-MS analysis was its poor response to laser interaction. When ablating a pressed powder pellet of NIST 1486 prepared at 0.6 MPa, a high proportion of > 1  $\mu$ m particles are generated. As a consequence, there is incomplete vaporisation of the larger particles in the plasma resulting in elemental fractionation and lower analytical precision when compared to NIST glasses. Hare et al. (2011) reports a similar problem when using NIST 1486 though they attribute their lower precision to sample heterogeneity. Whatever the reason, this material is best used in combination with another SRM. A promising alternative to NIST 1486 is NIST 1400. This is certified bone ash and when sintered at 2 GPa and 700 °C creates an ultra-dense pellet with excellent ablation performance. Using such material, Balter et al. (2008) reports, for example, a within-run precision for Sr/Ca of 1% by LA-ICP-MS. A more reliable matrix-matched standard for dental tissue analysis is clearly needed. Accuracy in laser ablation however is not solely determined by the matrix but is also a function of the laser-operating conditions (wavelength, pulse width, pulse energy). Short wavelength excimer lasers have proved very effective in minimising matrix effects since all matrices tend to absorb with similar efficiency (Jackson, 2008). Given that the concentration values obtained for Zn, Mg, Sr and Pb in NIST 1486 (external unknown) were within 10–17% of the certified values, we are confident that our use of NIST glasses for calibration is acceptable.

Although the ArF excimer laser used in this study is capable of producing much smaller ablation spots, a pit diameter of 100  $\mu$ m was selected to yield sufficient counts/sec/ppm Pb for the signal to be significantly in excess ( $\times 3$ ) of the background count rate. For the other elements (present at considerably higher concentrations) the spot size could have been reduced to 20  $\mu$ m or less without compromising detection limits.

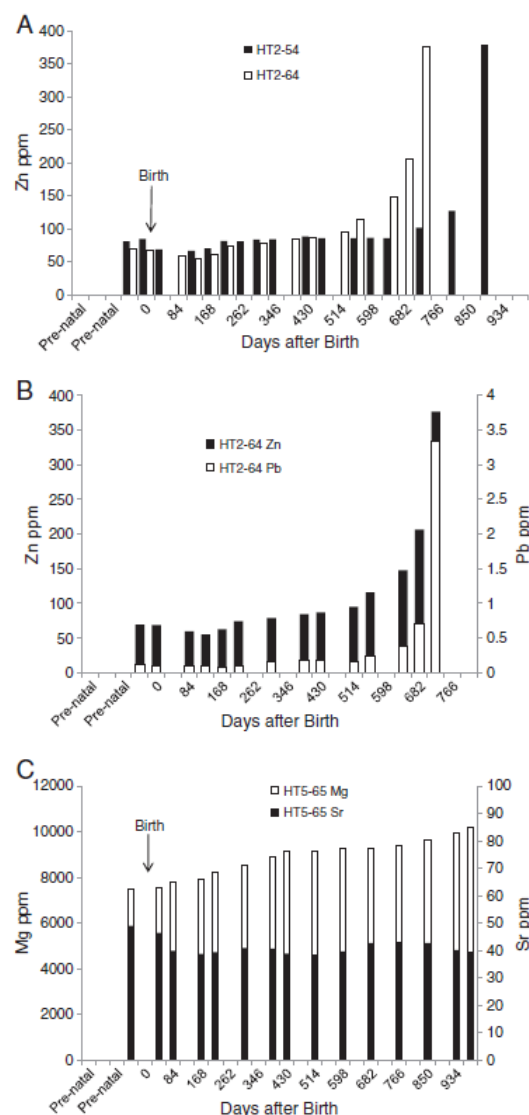
### 3.2. Histological analysis

Histological analysis was performed using an Olympus BX51 microscope with a Q-Imaging Micropublisher 3.3 RTV camera and Improvision Openlab 5.0.2 image analysis software. The ages sampled by each ablation pit were determined by identifying the neonatal line and measuring the distance from it to the edge of the first ablation pit. This distance was divided by the mean daily rate of dentine secretion as determined by the mean distance between daily growth increments in the same region; the von Ebner lines (Dean, 1995). This gave the number of days and the age from birth to the edge of the pit. In a similar manner, the mean rate of dentine secretion was determined alongside the pit to yield the number of days sampled and the age at the leading edge of the pit. The process was then continued along the transect until the ages sampled by each pit were determined. From these measurements we concluded that a 100  $\mu$ m ablation pit represented on average 42 days of dentine growth. Accordingly, a series of age ranges of 6 weeks duration were constructed allowing the LA-ICP-MS elemental data to be assigned a chronological age. A similar strategy was followed to determine the ages sampled by each ablation pit in the enamel, using daily cross striations. A detailed description of the histological analyses will be presented elsewhere (Dirks et al. *in prep*).

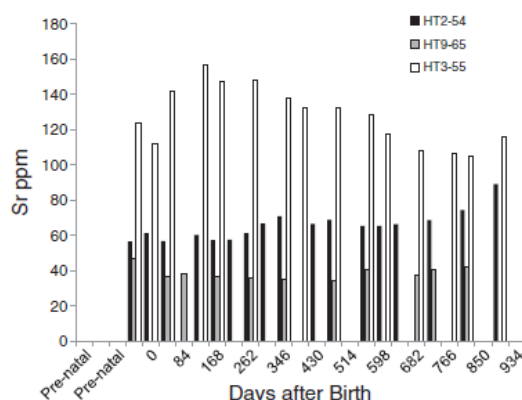
### 4. Results

Only the dentine analyses are reported here. Of the 19 teeth analysed, 5 have been excluded from discussion because the longitudinal sections were cut oblique to the ideal plane through the dentine and pulp horns and did not intersect the pulp cavity (see Table 1). To test the extent of within-tooth trace element variation, 3 to 5 ablation

transects were made across two pairs of teeth (HT1–54,55 and HT11–64,85), orientated to follow dentine tubules from the EDJ to the pulp cavity, from all regions within the crown (Fig. 1b). From these results and associated histological data it was evident that individual transects corresponded to different post-natal time spans. For HT11–85 the time span for dentine ranged 766 to 1018 days after birth. Nevertheless, irrespective of the time span, the overall



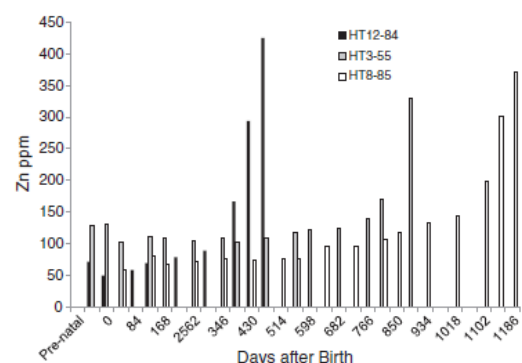
**Fig. 2.** a. Graph illustrating the excellent agreement for Zn concentrations in dentine for paired molars (child HT2). The time adjusted concentration profiles are very similar and independent of the age of final dentine secretion. Along the laser transect for HT2-54, the final dentine layer was secreted approximately 160 days after the corresponding layer in HT2-64. b. Graph showing the sharper increase in Pb concentration relative to Zn on approaching the pulp cavity for ablations along the same transect. All data are time adjusted. c. Graph demonstrating the contrasting behaviour of Mg and Sr during the secretion of dentine over a period of 970 days after birth for child HT5 (see text for details).



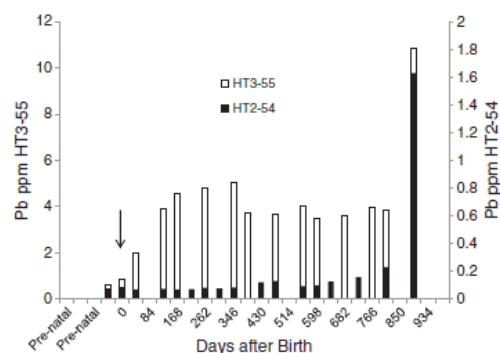
**Fig. 3.** Contrasting time adjusted Sr concentration profiles for three individual children illustrating the potential of laser ablation analysis to assess differences and changes in nutrition and diet over the first few years of early childhood.

concentration levels and concentration-time profiles (A, B and D) remained similar and highly correlated for each tooth (Fig. 1c). We decided therefore that for the remaining teeth only one transect was required to demonstrate the magnitude and temporal changes in trace element concentration; a conclusion reinforced by the agreement between tooth pairs. Table 3 summarises the maximum and minimum values for Pb, Zn, Sr and Mg in dentine for each tooth or pairs of teeth. Maximum values for secondary dentine, where analysed, are also shown.

Previous researchers (Arora et al., 2006; Humphrey et al., 2008; Kang et al., 2004) when reporting trace element analyses of dentine and enamel by LA-ICP-MS have erred on the side of caution, by normalising ion intensities to calcium. This avoids the problem of not knowing the absolute Ca concentration and/or within-dentine Ca variation. In this study, for example, the within-dentine  $^{44}\text{Ca}$  intensity variation (RSD 6%,  $n=15$ ) from the EDJ to the DPC for HT3-55 is too small to account for the corresponding RSD variations in ion intensities for  $^{208}\text{Pb}$  (59%) or  $^{66}\text{Zn}$  (42%). Moreover, though published Ca values for dentine in deciduous and permanent teeth differ by several wt.% (Arnold and Gaengler, 2007; Keinan et al., 2006; Ohmori, 1961), the relative uncertainty is small compared to the within-tooth and between-child differences in trace element concentrations



**Fig. 4.** Time adjusted Zn concentration profiles for 3 individual children showing the distinctive rise in concentration on approaching the pulp cavity. Note that the mean and maximum values in dentine differ very little between children, presumably in response to strong homeostatic control.

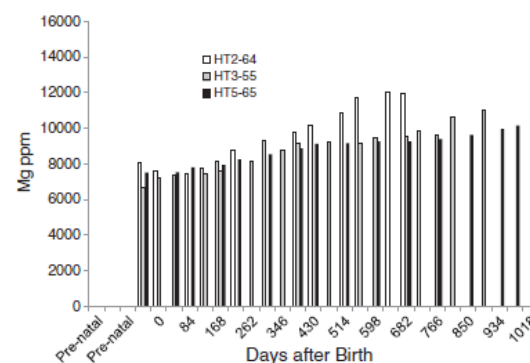


**Fig. 5.** Graph showing highly contrasting levels of total lead in dentine and corresponding time adjusted concentration profiles. The profile for HT3-55 is closely replicated in deciduous molar HT3-65 (not shown). Child HT3 also shows anomalously high levels of Sr compared to other children (see Fig. 2c).

(several orders of magnitude for Pb). We thus consider our measured trace element variation to be robust and to allow easy comparison with previously published data, we have retained the value of 26.5 wt.% Ca used by Arora et al. (2006). If in future there is consensus that the accepted mean Ca value for dentine is different, then the concentrations presented here can be recalculated accordingly.

In Figs. 2 to 6 the results are displayed graphically as time-concentration profiles. For clarity, the measurement error for each sample point is not shown. Selection was made to highlight the following deductions.

- Agreement for all elements for all pairs of teeth (Fig. 2a). This applies to combinations of both 1st and 2nd deciduous molars and is strong evidence to support the contention that position within deciduous dental arch is not an overriding complication in the interpretation of dentine data.
- Profiles for Pb and Zn display a systematic rise in concentration at or close to the DPC (Fig. 2b). This is evident in each and every tooth examined, including sections where the pulp cavity was separated from primary dentine by a thin layer of secondary dentine. The only exceptions being those samples for which longitudinal sections did not intersect the DPC (Table 3). For Pb, the rise is demonstrably sharper than for Zn. Values for Pb and Zn in secondary dentine are consistently



**Fig. 6.** Graph illustrating the monotonous rise in Mg concentration from birth to the cessation of primary dentine secretion.

higher than the corresponding highest values in contiguous primary dentine (Table 3).

- (iii) Sr shows either little or no change in concentration with time (excluding HT3). Mg differs from the other elements in displaying a steady and progressive increase in concentration from the EDJ to the DPC soon after birth (Fig. 2c).

## 5. Discussion

To facilitate the discussion and provide a context for interpretation we have used the following simplified time frame of deciduous tooth formation.

After early pre-natal differentiation of enamel and dentine, dentine continues to be laid down by odontoblastic cells that line the pulp cavity. These act as biological channelways in controlling the transfer of mineral ions from the blood plasma to the sites of dentine secretion. Dentine is first secreted as a layer of unmineralised matrix which forms a collagenous framework called predentine. This varies in thickness (10–30  $\mu\text{m}$ ) and contains dense clusters of apatite nanocrystals (<5 nm) (Nanci, 2008; Linde, 1992) which aggregate to form calcospherites. Gradually, by coalescence of these clusters, progressive growth of the apatite crystals and a decrease in collagenous material, the predentine mineralises into primary dentine in what is known as globular mineralisation. Linear mineralisation may also occur when the mineralising front appears as a line rather than as a scalloped edge. As apatite maturation proceeds, the odontoblasts migrate progressively inwards leaving a characteristic architecture of open tubules, marking the trace of the odontoblasts, embedded in a matrix of nanocrystalline platelets of apatite (30–50 nm) and about 20% organic material. For a typical deciduous tooth there are approximately 24,000 tubules/ $\text{mm}^2$  (Schilke et al., 2000). The secretion of dentine can therefore be described as a front of mineralisation linked to an inwardly migrating layer of unmineralised predentine. After a period of sustained dentine secretion there is then a hiatus during which root formation takes place followed by the formation of secondary dentine. At no stage does mineralisation lead to the occlusion of dentine tubules 'dental sclerosis' which implies that the main phase of mineral deposition is completed in close proximity to the predentine layer and does not contribute appreciably to nanocrystal growth elsewhere within the earlier formed dentine. Of the various processes invoked for the localisation of mineralisation, the evidence favours cellular control by the odontoblasts (Nanci, 2008; Linde, 1992). The final stage of deciduous tooth development is resorption of the root and shedding of the crown.

From work on the maturation of synthetic bioapatites, Cazalbou et al. (2004b, 2005) conclude that Ostwald ripening (McNaught and Wilkinson, 1997) and dissolution-precipitation mechanisms (excluding the fluoridation of dental enamel) are relatively slow and are not appropriate on a biological timescale. Instead, they propose a model of a structured hydrated layer 'a non-apatitic environment', located at the surface of apatite nanocrystals, containing easily exchangeable mobile ionic species. With maturation and growth of the nanocrystals, the non-apatitic domain decreases with respect to the bulk apatitic domain. During this process, ions in the hydrated layer are irreversibly incorporated into the apatite lattice or enriched in the residual hydrated layer. Maturation rates, based on the synthesis of carbonated apatite, lead to a stable end phase within 20–30 days. Boanini et al. (2010) have also drawn attention to the fact that not all trace elements in nanocrystalline carbonated apatite are ion substitutions in the apatite lattice but some may be absorbed onto the crystal surface (i.e. doped ions).

When considered collectively, the complementary hypotheses of hydrated surface layers and ion doping, as a function of crystal size, offer a possible explanation for changes in the concentration of trace elements in dentine during mineralisation. Using these

experimental observations, the following four sections detail the variation of Sr, Zn, Pb and Mg in dentine for 18 deciduous molars.

### 5.1. Strontium

For the majority of teeth there is little apparent variation in Sr from the DEJ to the DPC, including secondary dentine (Table 3). There are however notable differences in the time averaged mean concentrations of Sr between children (Fig. 3). Child HT9 maintains a Sr level of 38 ppm ( $\pm 3$  ppm  $1\sigma$ ) between birth and the cessation of primary dentine formation, whereas child HT2 displays a slightly more elevated, variable level of 65 ppm Sr ( $\pm 8$  ppm  $1\sigma$ ), with child HT3 maintaining a very elevated level of 135 ppm Sr ( $\pm 13$  ppm  $1\sigma$ ). These changes are most likely a response to diet and whether or not the child was breast fed or weaned on proprietary milk products (Humphrey et al., 2008). Lacking such critical information, we are unable to differentiate between the various controls. Overall, the profiles for Sr are in marked contrast to those of Zn, especially the absence of increasing enrichment in proximity to the pulp cavity (see later). Where there are major changes in Sr concentration, they tend to be sharp and completed within a relatively short time interval (e.g. child HT3 shows an increase in Sr from birth to 126 days of 50 ppm). One possible explanation for these patterns is the close similarity in ionic radius of Sr (0.132 nm) and Ca (0.114 nm). By substituting very easily for  $\text{Ca}^{2+}$ , the  $\text{Sr}^{2+}$  ion would be strongly and rapidly stabilised in the ordered apatite lattice of the growing nanocrystal. Sr may thus be regarded as controlled primarily by ion substitution and therefore a sensitive indicator of blood plasma levels. Secondary dentine, where present, is not enriched in Sr relative to primary dentine.

### 5.2. Zinc

Unlike Sr, Zn is homeostatically controlled and one would not expect major postnatal changes in concentration with age. For the majority of tooth profiles this is true. Excluding child HT3, time averaged mean concentrations of Zn vary from ~50 to 90 ppm but increasing to 300–400 ppm within 200–300  $\mu\text{m}$  of the DPC (Fig. 4). In this respect Zn differs substantially from Sr. The smooth, systematic increase in concentration on approaching the pulp cavity occurs irrespective of the age of the final dentine layer or the age of the child at the time of tooth extraction (Fig. 4). For HT12–84 primary dentine was completed at ~514 days, for HT3–55 at ~934 days and HT8–85 at ~1186 days. This strongly suggests that the process controlling Zn incorporation into carbonated apatite is different from that of Sr and is a function of dentine growth. X-ray absorption studies on bone, a close carbonated apatite analogue for dentine, have demonstrated that Zn atoms are localised at the surface of apatite nanocrystals and not in the apatite structure (Bazin et al., 2009). Since the surface area/volume ratio decreases with increasing crystal size, there will be a proportional decrease in the Zn/Ca ratio away from the predentine layer; in good agreement with the observed dentine profiles. We suggest therefore that the concentration of Zn in dentine is primarily a response to homeostatic control but modified by ion doping and absorption processes concomitant with crystal growth. Because ion absorption is a function of the available surface area, rapid changes in the Zn content of dentinal fluid, and by inference blood plasma, are probably smoothed out. A preference for surface absorption as distinct from ion substitution is also in agreement with the smaller ionic radius of Zn (0.088 nm) (Boanini et al., 2010). Secondary dentine appears to amplify and continue the apparent enrichment trend described earlier (Table 3). For example, the Zn concentration in secondary dentine for tooth HT13–84 is more than twice that of the contiguous primary dentine. This could be due to a slower rate of dentine secretion allowing a switch from ion absorption to ion substitution or a decrease in crystal size.



### 5.3. Lead

Lead shows characteristics of both Sr and Zn. Like Sr, the profiles for Pb are relatively flat for most children and, excluding dentine close to the pulp cavity, show no marked changes in concentration with age. Time averaged means for primary dentine (excluding values close to the DPC) differ by only a factor of 2 to 3 for the whole cohort (max ~0.3 ppm; min ~0.1 ppm). By contrast, the time averaged Pb concentrations for child HT3 are extremely anomalous and are 10 times greater than those of the other children, implying a dramatically higher level of environment lead exposure (Fig. 5). Furthermore, HT3 displays rapid, reproducible changes in Pb that can be traced in both deciduous molars (HT3–55,65) from birth to the DPC. Of course, we recognise the danger in over extending our interpretation based on such a small cohort and two profiles. Nevertheless, though lacking X-ray spectroscopic verification, we tentatively suggest that Pb, having an ionic radius (0.130 nm) similar to Ca (0.114 nm) and with profiles that record rapid changes in concentration, is controlled primarily by ion substitution. This does not necessarily preclude some degree of surface absorption since there is a weaker but definite enrichment in Pb on approaching the pulp cavity. Typically, Pb concentrations rise sharply to ~2–6 ppm within 100–200 µm of the DPC. [N.B. Because the gradient in Pb concentration at the DPC varies with a few 10's of microns, the position of the ablation pit (diameter 100 µm) is critical in estimating the maximum concentration.]

### 5.4. Magnesium

In general, the profiles for Mg differ from those for Sr, Zn and Pb in showing a steady rise in concentration from ~7000 ppm at the EDJ to ~10,000 ppm at the DPC (Fig. 6). The rate of increase is variable and in one case (HT11) the Mg concentration decreased with age after 1 year (from ~7000 ppm to ~2000 ppm) on all 5 ablation transects. We are unable to find any analytical explanation for this anomalous behaviour and the reversal was not observed on corresponding Pb, Zn or Sr profiles. Lacking published data for changes in plasma Mg levels during early childhood we are unable to comment further. Though not observed for all teeth, there is a tendency for a sharp decrease in Mg immediately adjacent to the DPC (100–200 µm: equivalent to ~1–2 months) suggesting that immature dentine is perhaps Mg-deficient (see Fig. 6: HT2–64). Some uncertainty exists as to the exact localisation of Mg in biological apatites (Cazalbou et al., 2004a) although the similarity in ionic radius between Mg (0.086 nm) and Zn (0.088 nm) would tend to favour control via ion absorption. Whether the temporal changes we observe are a response to plasma Mg concentration, dentine composition or are related to the kinetics and growth mechanisms of nanocrystalline carbonated apatite remains to be proven.

Trace element enrichment close to the pulp cavity has been reported by many other researchers, notably Rabinowitz et al. (1993), Thuy et al. (2003), Kang et al. (2004), Arora et al. (2006), Richter et al. (2011), Arora et al. (2011) and Hare et al. (2011). Arora et al. (2006) in agreement with Rabinowitz et al. (1993) suggested that Pb enrichment was possibly due to the close juxtaposition of odontoblasts and blood vessels, allowing a higher rate of exchange between blood and dentine. This hypothesis was later invoked by (Arora et al., 2011) to account for higher levels of Mn in circumpulpal dentine adjacent to the pulp cavity in naturally shed deciduous incisors. More recently, Hare et al. (2011) have very elegantly demonstrated, using LA-ICP-MS bio-imaging, an enrichment of Pb and Zn around the margins of the pulp cavity in naturally shed deciduous incisors. Whilst the analysis of fluorine in dentine is not possible by ICP-MS, electron microprobe (Richter et al., 2011) and specific ion electrode (Thuy et al., 2003) studies have shown a major increase in fluorine concentration in close proximity to the pulp cavity. In the case of Thuy and co-workers, the illustrated fluorine enrichment profiles are remarkably

similar to those of Zn and Pb in the present study, although the authors make no reference to secondary dentine which would have been well developed in permanent premolars in subjects 13–22 years of age.

This study focussed on the need to acquire data for very low concentrations of Pb; hence the use of 100 µm diameter ablation pits. However, as described in Section 3.1, given the higher concentrations of Sr, Zn and Mg, satisfactory signal/background ratios could have been obtained using a 20 µm diameter laser beam, equivalent to a dentine secretion interval of <10 days.

### 5.5. Dentine as a biomarker of lead exposure history

From the foregoing observations and the excellent reproducibility demonstrated by pairs of deciduous molars, the evidence strongly supports the assertion that dentine carries a record of plasma lead concentrations. As mentioned in Section 3.2, to unlock this information, each laser ablation point was assigned a real time interval based on the number of days before and after birth. From this we deduce that:

- (i) The steep increase in Pb concentration on approaching the pulp cavity 'pulp proximity enrichment' is not a function of increasing plasma Pb concentration or age of the dentine. The most likely cause is nanocrystal maturation.
- (ii) There is a finite time interval 'duration of mineralisation' between predentine formation and a stabilised Pb concentration. Thus changes in plasma Pb concentration with time 'history of exposure' can only be inferred from changes in dentine Pb concentration after peak mineralisation (i.e. within a stable baseline). There is therefore a finite time delay between the environmental exposure event and its fixed expression in primary dentine. At present we do not have the necessary data to quantify this duration. The large diameter of the laser ablation pits (100 µm) combined with the separation between pits (100 µm) is inadequate to accurately detect small scale changes in lead concentration close to the pulp cavity. Work is in progress to use a smaller diameter laser beam in conjunction with a higher sensitivity mass spectrometer to detail trace element variation within this critical zone.
- (iii) In the absence of major temporal changes in dentine Pb, time averaged means can be used as a comparative measure of the relative degree of exposure for a given child. The higher the background environmental lead, the higher the time averaged dentine lead. Since the concentration of Pb close to the DPC is similarly a reflection of plasma Pb, these values if used judiciously might also provide comparisons of relative exposure.

Point (iii) poses the important question: Can dentine lead be used as an empirical measure of cumulative childhood lead exposure? Being non-invasive, it has potential merit and compares favourably with *in vivo* bone lead studies (Ambrose et al., 2000; Hu, 1998). Unlike bone however, there is no evidence to indicate a turnover of dentine lead (Gulson, 1996). Nor do teeth store sufficient amounts of lead as to pose a longer term health risk.

Secondary dentine remains an unresolved problem. As shown in Table 3, it is anomalously enriched in Pb compared to primary dentine and in permanent teeth, where it is better developed, displays an incremental layered structure. Zn also shows a modest enrichment (30–50%). For Mg and Sr, secondary dentine is indistinguishable from primary dentine and according to Nanci (2008) has the same ratio of mineral to organic material. Due to the hiatus in dentine formation during the period of root formation, secondary dentine cannot be considered a simple continuation of primary dentine secretion. Odontoblast activity, in controlling the transfer of mineral ions to the predentine layer, is temporarily suspended. On resumption of activity the incorporation of trace elements into the carbonated apatite is evidently different. Unfortunately, lacking data for the timing of

secondary dentine formation or its nanocrystallinity, we can only speculate that the enrichment of Pb (and Zn) is due either to slower growth kinetics or crystal size. There is however a third factor to consider; namely root resorption prior to tooth shedding. The natural process of root dissolution releases previously stored lead (Arora et al., 2004) directly into the blood plasma. By analogy with primary dentine, such additional lead would be rapidly incorporated into secondary dentine. Comparison of the Pb levels in primary and secondary with respect to exposure history is therefore difficult to interpret and further high spatial resolution analysis across the primary-secondary dentine interface is clearly warranted.

The ability to resolve time differences of ~42 days permits an exciting insight into patterns of pre- and post-natal lead exposure. As demonstrated by Arora et al. (2006), using the neonatal line as a fixed point in time, it is possible to distinguish between plasma lead levels corresponding to the mother's blood lead during term and the child's ambient exposure to lead immediately after birth. A sense of change can be seen in child HT3 where a pre-natal dentine Pb concentration of ~0.6 ppm increases to ~0.9 ppm within 42 days of birth. Although these values are low, the ability to detect measurable differences indicates the potential of LA-ICP-MS to provide information on lead exposure during and after foetal development. Whilst this paper does not address health issues linked to lead exposure, one of the most important goals of our long term research programme is to validate the use of dentine lead as a proxy for BPb. However, we recognise that this will require teeth from children living in more polluted areas and access to serial blood samples. Several studies have used dental tissue data to infer BPb levels but, as emphasised by Grobler et al. (2000), the results are often difficult to compare because different parts of the tooth were analysed and the tests lacked crucial information that would allow unambiguous temporal correlation with blood samples. By successfully using histological criteria to determine an age for each dentine micro-analysis, we believe our study brings the development of a retrospective BPb biomarker one step closer. Finally, although we have emphasised the reconstruction of exposure history, a true time frame also facilitates a more reliable estimate of cumulative exposure.

## 6. Conclusions

Lead analyses for 19 deciduous molars combined with detailed histological work to establish the age of the dentine confirm that primary circumpulpal dentine can be confidently used to reconstruct the history of early childhood lead exposure. Using a laser beam of 100 µm diameter, the time span of each ablation point corresponded to an average of 42 days of dentine growth. Complementary data acquired for Zn, Mg and Sr have proved very informative in identifying the mechanisms controlling the uptake of Pb into dentine, and suggest that these controls are linked to the special physical and chemical properties of nanocrystals and nanocrystal growth. For elements with higher dentine concentrations than Pb, a spatial resolution of 10 µm is well within the analytical sensitivity of quadrupole mass spectrometry.

Between-tooth differences for paired deciduous molars were found to be small and, although within-tooth dentine variation can be detected, robust and reproducible differences in exposure to Pb can be quantified using a single, well positioned, laser ablation transect on longitudinal sections. Interpretation must however distinguish between primary and secondary dentine and allowance made for a possible time difference (small but as yet unquantified) between pre-dentine formation and establishment of a stabilised Pb dentine signal. Unlike enamel which ceases to form before the tooth has erupted into the oral cavity, dentine formation in deciduous teeth is more continuous and affords an unbroken record of first few years of post-natal history.

To achieve the longer term goal of using dentine as a proxy for BPb it is important that experimental designs incorporate procedures for determining the age of the dentine, to permit unambiguous temporal correlation with blood samples. Moreover, since blood Pb has a short half life and that close to the pulp cavity Pb displays a very high concentration-time gradient, small errors in assigning an age to the dentine can lead to large errors in estimating BPb levels. We contend that in the absence of a true time frame, serious miscalculations may arise in using Pb analyses for dental tissues as proxies for BPb.

Whilst this study focussed on exposure to Pb, the multi-element capability of LA-ICP-MS opens up potential avenues of research in epigenetics where changes in both toxic and essential elements during early childhood can have important health outcomes later in life. This study also signals future challenges for histological research. Of particular interest would a better understanding of the time gap between the cessation of primary dentine formation and secretion of secondary dentine. Use could then be made of the information stored in secondary dentine and thus further extending the historical record of childhood exposure.

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## NEWS

# Developmental Variation of the Primate Dentition: The 2011 AAPA Symposium in Honor of Don Reid

On April 15<sup>th</sup>, 2011, a special AAPA symposium was held in Minneapolis, Minnesota to celebrate Don Reid's influential contributions to the field of dental histology. Twenty presenters gathered to showcase state-of-the-art research on dental development and structure, share treasured stories, and express their gratitude for their recently retired colleague (Fig. 1). We begin this review with a brief history of Don's remarkable 50-year career, followed by a summary of the research presented in Minneapolis.

In 1961, at the age of 16, Don took a position as a histology technician in the Anatomy Department of Kings College (United Kingdom). His early work with Howard Tonge at the Dental School included assisting in groundbreaking research on calorie and protein deficiency effects in developing pig dentitions.<sup>1-3</sup> During this time, Don also began working with David Beynon at Newcastle University to investigate enamel mineralization.<sup>4,5</sup> In the early 1980s, along with David Beynon, Alan Boyde, Timothy Bromage, Chris Dean, Lawrence Martin, Peter Shellis, and Bernard Wood, Don's careful histological labors helped to usher in a revolutionary new approach to understanding the evolution of human growth and development.<sup>6</sup> Don, David, and Chris also began to document incremental dental development in living great apes and humans,<sup>7-9</sup> as well as in the Miocene hominoid genus *Proconsul*.<sup>10</sup> Until quite recently, these studies represented the only available comparative histological data, illuminating important aspects of hominoid life history and systematics. In 2000, Newcastle University awarded Don a Ph.D. for his prolific research on primate dental development, followed by a 2003 appointment as a Lecturer in recognition of his invaluable role at the Dental School.

Perhaps Dr. Reid's most significant contribution, in addition to his patient mentorship and collaboration with dozens of scholars, has been his

seminal research on developmental variation in modern humans. Thousands of hours of histological preparation and analysis have demonstrated that modern human populations do not all develop their teeth identically,<sup>11,12</sup> facilitating more precise comparisons with fossil hominins and nonhuman primates.<sup>13-16</sup> Studies of modern human dental development have also established that external tooth crown increments (perikymata) are not laid down at a uniform rate from the beginning to end of crown formation.<sup>11,17</sup> These results have redefined the aging of linear enamel hypoplasias and serial isotopic samples of tooth crowns, calling into question previous bioarcheological and isotopic studies that assumed spatial and temporal equivalence. Moreover, Reid and Ferrell<sup>18</sup> demonstrated a novel inverse relationship between the number of long-period growth lines (perikymata, Retzius lines) and their intrinsic temporal periodicity. This relationship has helped to contextualize the marked degree of variation in long-period line numbers in human populations and has had important implications for studies of fossil hominins.<sup>13</sup>

A survey of research presented during the 2011 symposium underscores how the field continues to evolve, due in part to Don's important contributions, and the growing connections among dental histologists. All 20 participants are linked with Dr. Reid by no more than three degrees of academic separation; in this field nearly everyone is a collaborator, mentee, or one of Don's "grand-students" (Fig. 2). Presentations focused on dental studies of fossil primates, living nonhuman primates, and modern humans.

Papers on fossil dental development ranged from the Eocene adapid *Leptadapis magnus* to *Homo neanderthalensis*, often emphasizing relationships among dental development, life history, diet, and systematics.

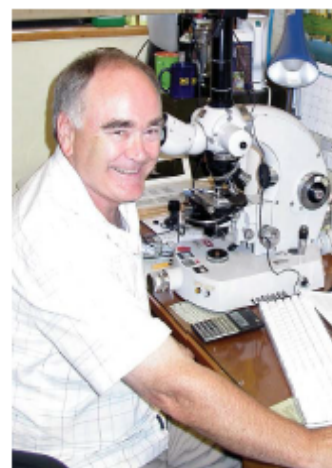


Figure 1. Don Reid at work with his beloved microscope. Photo courtesy of Wendy Dirks. (Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).)

Wendy Dirks (Newcastle) and colleagues showed that *Leptadapis magnus*, which had morphological crown features indicative of folivory, also had characteristics of rapid molar development associated with folivory. Plio-Pleistocene hominins were represented by two papers. Rodrigo Lacruz (University of Southern California) and colleagues reported differences in cusp and crown base areas between *Australopithecus anamensis* and *Australopithecus afarensis* molars, adding additional morphological weight to taxonomic distinctions. Paul Tafforeau and co-workers, represented by coauthor Joane Pouech (European Synchrotron Radiation Facility, Grenoble) presented synchrotron imaging data from juvenile *Paranthropus robustus* and *Homo* sp. dentitions from Drimolen, South Africa (2.0-1.5 mya). Short formation times were found in *Paranthropus* molar cusps as compared to *Homo*, underscoring developmental differences between these two coexisting genera. Tanya Smith (Har-





Figure 2. Participants at the AAPA 2011 symposium in honor of Don Reid. From left to right: Charuwan Manmee, Halszka Glowacka, Akiko Kato, Joane Pauich, Robin Feeney, Jacopo Moggi-Cecchi, Kierstin Catlett, Laute Godfrey, Russell Hogg, Debbie Guatelli-Steinberg, Julia Boughner, Rodrigo Lacruz, Sarah Martin, Sarah Holt, Bernard Wood, Daniel Antoine, Wendy Dicks, Don Reid, Simon Hillson, Justyna Miskiewicz, Daniel Green, Patrick Mahoney, Tanya Smith and Gary Schwartz. Photo courtesy of Brett Nachman and Daniel Green. (Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).)

vard) and colleagues discussed recent work on ontogenetic differences between Neanderthals and modern humans. By also using synchrotron virtual histology, they demonstrated that Neanderthals had shorter periods of dental development than modern humans, suggesting that Neanderthals may also have had more rapid life histories than modern humans.

Across the primate order, the pace of primate life histories is strongly correlated with the timing of dental development, brain size, and body size.<sup>19</sup> Gary Schwartz and Jay Kelley (ASU) summarized current perspectives on the relationships between age at molar eruption and other life history-related variables, suggesting that early hominins erupted their first molars at surprisingly young ages relative to wild apes. Daniel Green (Harvard) and colleagues demonstrated that great apes show broadly similar rates of enamel secretion and periods of crown formation, despite differences in crown size. Notably, large gorilla molar teeth form through rapid recruitment of enamel-forming cells, which may relate to their accelerated life histories.

The nature of relationships among dental development, somatic development, skeletal development, and life history is complex. Indeed, Kierstin Catlett (ASU) and col-

leagues showed that within the Indriid-Palaeopropithecoid clade, the timing of dental development is not related to species-level differences in brain and body size, and it is rapid relative to somatic and cranial growth. Factors such as diet and local ecology may be critical for understanding variation. Russell Hogg (Univ. Missouri) and colleagues presented complementary research demonstrating differences between haplorhines and strepsirrhines in the relationships among long-period line periodicities and life history-related variables (body mass, brain mass, and metabolic rate). The decoupling of dental and somatic growth has been known from the modern human literature for some time.<sup>20</sup> Julia Boughner's (Univ. Saskatchewan) paper provided an exciting glimpse into the molecular mechanisms that may underlie this independence. She contrasted gene expression in embryonic mouse mandibles from a normal strain with those from a "toothless" strain in which mandibular development proceeds normally but tooth development is arrested before the bud stage. In these mice, genes known to be involved in mandibular morphogenesis were expressed at normal levels, while most genes with known involvement in dental development were not.

In his highly anticipated comments, Dr. Reid called for additional research on variation in modern human dental development. Several symposium papers strived toward this goal. Fernando Ramirez-Rozzi (CNRS) and Marta Lahr (paper presented by Rodrigo Lacruz) studied dental growth in Australian aborigines, reporting crown formation periods at the long end of the known modern human range. Patrick Mahoney (Kent) and colleagues compared dental development in Fulbe pastoralists and Nso agriculturalists from Cameroon. While the diets of these populations differ significantly, which was supported by isotopic work, enamel development does not appear to differ.

Daniel Antoine (British Museum) and colleagues added to the limited histological record on crown initiation timing by examining post-Medieval children of known age. Until recently, data on modern human crown initiation ages have been derived from radiographic studies, which cannot detect the earliest stages of mineralization.<sup>5</sup> Interestingly, Antoine and colleagues documented earlier anterior tooth initiation ages in this sample than have been found in Europeans.<sup>9</sup> Sarah Holt (Ohio State) presented novel findings on enamel secretion rates in deciduous teeth from a contemporary Italian



population and a Roman archaeological site, which did not appear to differ between populations.

Debbie Guatelli-Steinberg and co-workers investigated the relationship between enamel extension rates, the rate at which enamel-forming cells differentiate along the enamel-dentine junction, and the distribution of perikymata on the lateral enamel surface of modern human teeth. They found a decline in the rate of extension that corresponds to a decrease in perikymata spacing. The study was motivated by the fact that different hominin species exhibit variation in perikymata distribution.<sup>21,22</sup>

While the preceding studies focused on normal tooth development, several papers addressed disrupted growth. Simon Hillson (UCL) and Daniel Antoine presented a carefully illustrated case study of enamel hypoplasia in an enigmatic post-Medieval individual. They documented a range of synchronous developmental defect manifestations that differed within teeth and would not have been evident from tooth surfaces alone. Justyna Miskiewicz (Kent) and Patrick Mahoney found a relationship between linear enamel hypoplasia (formed during childhood) and adult age at death in Medieval burials from Canterbury, UK. Higher status individuals had lower frequencies of enamel hypoplasias and later ages at death than did lower status individuals. Sarah Martin (Ohio State) and colleagues examined the relationships between crown formation time, fluctuating asymmetry, and developmental defects (linear enamel hypoplasias) in the canines of male and female gorillas and gibbons. They found that male gorilla canines, which grow over a longer period than females (or gibbons of either sex), show the highest degree of developmental disturbance, while monomorphic gibbons do not show sex differences.

The symposium also included cutting-edge research on modern human dental tissue structure and chemistry. Robin Feeney (Univ. Dublin) and colleagues reported differences in dental tissue proportions between male and female modern human molars. Males were found to have larger dentine and pulp volumes and enamel-dentine junction areas

than do females, leading to relatively lower average and relative enamel thickness. Charuwan Manmee (Newcastle) and colleagues presented a histologically informed assessment of lead levels in children from northern England. They found that lead levels occur at the same age when sampled along several transects in the circum-pulpal dentine, suggesting that only one transect is required to assess age-specific lead exposure in dentine.

The closing discussion was led by Bernard Wood (George Washington University), who related tales of working with David Beynon and acknowledging the critical "behind-the-scenes" role Don played in early studies of fossil hominin and living primate development. Bernard also highlighted something Don's colleagues and students know well: his openness and patience with the rest of us novice histologists is unparalleled. Beyond the significant scientific contributions he has made to the study of dental development, Don Reid's unflagging spirit of generosity and collegiality is an inspirational lesson for all evolutionary anthropologists.

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# APPENDIX IV: POSTER PRESENTATIONS

## 1. Lead levels in teeth as a measure of life-time lead exposure in children (Poster presentation, 23rd Annual Conference of the International Society for Environmental Epidemiology (ISEE), Barcelona, Spain, September 2011)



### Lead levels in teeth as a measure of life-time lead exposure in children

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#### Introduction

- Lead is used in the manufacture of paint, petrol, plumbing solders etc. Children often experience higher exposure and are more susceptible to deleterious health effects than adults, such as delayed growth and development and adverse behavioural effects. Blood lead is used to estimate body burdens but can only show current exposure. The acceptable blood lead level (BLL) in children is 10 µg/dl, however there is evidence of harmful effects below this level.
- There is a need for developing sensitive and specific biomarkers for chronic lead exposure to identify children and areas at risk for negative impacts of lead exposure

#### Materials and Methods

- Two deciduous molars each from 15 children aged 6-8 living in Northeast England were collected. Standard histological sections were analysed to determine the age at each sampling point using growth increments in enamel and dentine. Five ablation transects (A-E) with a minimum of 10 points per line were made across the surface of the section and the ages sampled by each ablation pit were determined. (Figure 1) Calcium normalised lead ratios ( $^{208}\text{Pb}/^{44}\text{Ca}$ ) were measured along transects across the tooth surface using Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS). Blood samples (5ml) were collected, and blood lead levels (BLLs) were measured using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Geographic Information System (GIS) was used to map lead intensities across the tooth surface, modifying the method of Humphrey et al (2008). The data were grouped into age intervals of 42 days, based on the mean time interval sampled. Lead intensities were plotted for each interval along each transect.

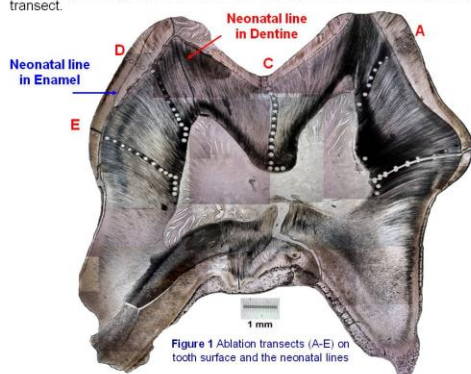


Figure 1 Ablation transects (A-E) on tooth surface and the neonatal lines

- The measurement to estimate age of each sampling point was as follows:
  - The distance (X and Y) from the neonatal line to the edges of each point (e.g. D1) were measured as shown in Figure 2
  - Cross striations in enamel, and von Ebner's lines in dentine were measured, and the mean daily secretion rate derived
  - The ages sampled for each ablation point (e.g. D1) was calculated by dividing the distances in µm (X and Y) with the mean of the cross striations or the von Ebner's lines, using the neonatal line as a reference

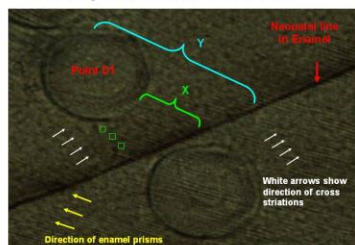


Figure 2 Measurement of the age at ablation point in postnatal enamel of primary tooth (40x)

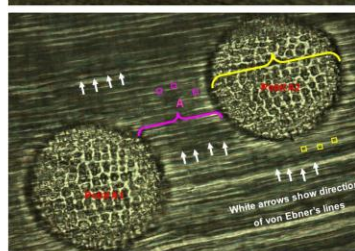


Figure 3 Measurement of the age at ablation point in postnatal dentine of primary tooth (40x)  
The age at point A2 is about 32-72 days after birth

#### What is the connection between teeth and lead?

- During development lead is incorporated into teeth as they form. Deciduous teeth are easy to collect and develop over a chronologically specific period, so a time line of formation can be identified. Teeth can therefore provide a 'time line' of lead exposure from in utero exposure through to current exposure. (Barbosa et al, 2005)

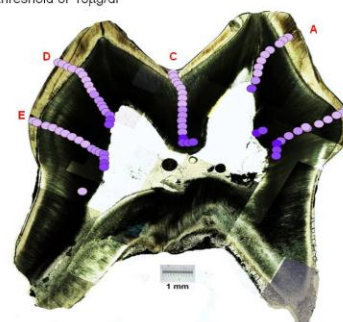
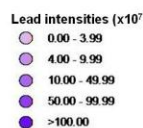
#### Aims

- To determine lead levels in different parts of the tooth
- To develop a method to assess long term lead exposure using tooth lead levels
- To explore the correlations between tooth and blood lead levels

#### Results

- Blood lead levels ranged from 0.5-6.8 µg/dl, and none of the children had a BLL above the WHO threshold of 10 µg/dl

Figure 4 Normalised lead intensities across tooth surface using GIS



- The mean daily secretion rate of enamel and dentine are shown in Table 1

Table 1 The mean daily secretion rate of enamel and dentine

Growth lines	Mean	SD	Min	Max
Cross striations in enamel (µm)	3.6	0.34	2.1	4.9
von Ebner's lines in dentine (µm)	2.9	0.30	1.4	4.8

- In the initial four teeth examined, lead intensities in primary dentine ranged from 1.65-95.96 (median=4.45). Standard sigma errors ranged from 0.15-13.88 (median=2.08). Lead intensities and age interval in one tooth from each child are shown in Figure 5. A similar pattern of relatively low lead intensity across the primary dentine followed by a rapid increase near the pulp cavity was observed in both teeth from each child.

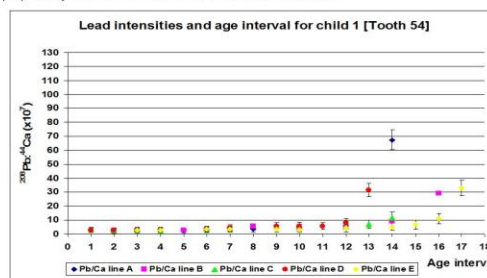


Figure 5 Lead intensities and age interval in one tooth from child 1

#### Conclusions

- Preliminary results indicate that normalised lead intensities in primary dentine are consistent between teeth from the same child, and at the same age within each tooth, indicating that the history of lead exposure may be determined using one ablation transect in primary dentine in one tooth per child. We will apply this new method to a pre-existing collection of deciduous teeth from 69 children living in Newcastle upon Tyne to investigate the environmental and social variables influencing long term lead exposure.

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## 2. Dentine lead levels in children in Newcastle upon Tyne

(Poster presentation, 23rd Annual Conference of the International Society for Environmental Epidemiology (ISEE), Barcelona, Spain, September 2011)



### Dentine lead levels in children in Newcastle upon Tyne

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#### Background

Lead still causes health concern in many areas of the world (Barbosa *et al.*, 2005), there is therefore a need to better characterise lead exposure especially in children. Newcastle upon Tyne has a long history of lead smelting in the city and lead mining in surrounding areas and is therefore highly suitable for such studies.

#### Aim

This study aimed to use dentine lead levels (DLLs) to explore determinants of lead exposure in children living in Newcastle upon Tyne. (Tooth fairy study)

#### Methods

The cohort consisted of 69 children aged 5-8 years who donated upper incisor teeth and whose parents answered a questionnaire. Figure 1 shows the city of Newcastle upon Tyne areas and indicates areas of the previous Byker soil lead study. To quantify DLLs, primary dentine from 4-6 ablation points per tooth were measured using Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS). The distribution of DLLs for each child is shown in Figure 2. To identify determinants of early life exposure, associations between DLLs and demographic characteristics, residential areas, dietary behavior, socio-economic status, hand to mouth behavior, occupational activities of household members, health conditions and soil lead levels were assessed.



Figure 1 The city of Newcastle upon Tyne map

#### Results

- Of the 69 children, 52% were girls, most were aged 6 years.
- DLLs ranged from 0.06-0.77ug/g, (Mean±SD = 0.25±0.16). In univariate analysis, significant determinants of DLL were methods of cleaning floors in the house; growing fruits/vegetables in the garden; and tap water consumption. In multivariate analysis, no significant relationship between determinants of lead exposure and DLLs was found.
- Soil lead levels from the areas of Byker incinerator were available as an additional potential determinant of DLL (Rimmer *et al.*, 2006) with soil lead levels ranging from 40 to 4,134mg/kg (mean±SD=350.46±472.34). 20% of samples exceeded the UK soil guideline values of 450mg/kg. A BGS study of sediment lead levels reported concentrations in sediments from 13 to 1,939 mg/kg (mean±SD=133±201.6) However, sampling points from these studies had only a small overlap with the recruitment area of the Tooth fairy study. (Figure 3)

#### Results

Newcastle tooth fairy study: dentine lead levels by child, in ascending order of median (n=69)

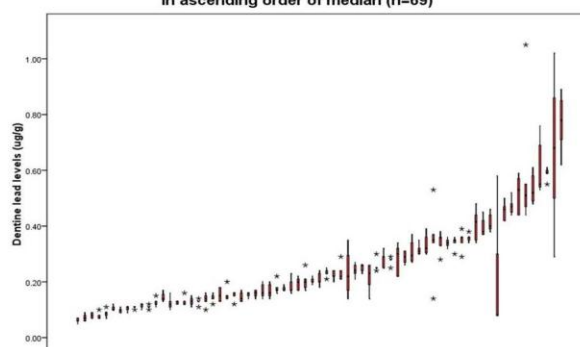


Figure 2 Distribution of dentine lead levels for each child

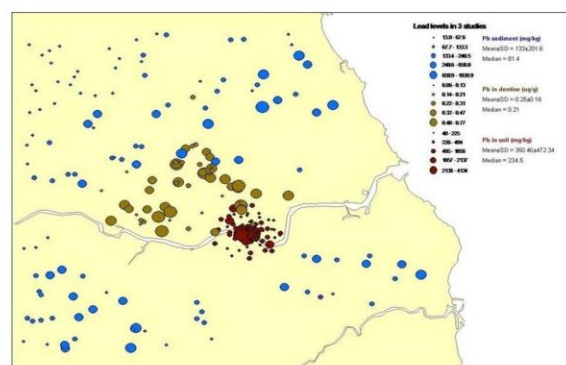


Figure 3 Lead levels in dentine, soils and sediments

#### Discussion and Conclusion

We did not find a relationship between determinants of lead exposure and DLL in Newcastle in spite of high lead levels in soil in areas near the residences of children, although we were not able to explore individual soil exposure specifically. This is in contrast to studies from other parts of the world. The findings may point towards less bioavailability of lead via the soil exposure pathway in this city. Determinants such as age of housing, which is often reported as important for studies of blood lead in the United States was also not relevant in this setting. In the light of the current evidence on the toxicity of lead in children further work to characterise lead exposure in the UK is still required. Dentine lead offers the opportunity to study histories of exposure in greater detail and should be developed further

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